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DETERMINATION OF ORGANIC ACIDS

III. NOTE ON THE USE OF THE ISOAMYL ETHER-WATER SYSTEM IN THE PARTITION METHOD

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The partition method for the quantitative determination of fatty acids in mixtures employs the differential distribution of the acids between either isopropyl ether and water (1) or ethyl ether and water (2). In the present paper necessary data are presented in tabular and graphical form for the use of the system of isoamyl ether and water. These data enable quantitative determinations to be made of the relative percentages of two fatty acids in a mixture. For a discussion of the principles underlying the partition method he reader is referred to previous papers (1 and 2). In the present paper only brief mention will be made of the details of procedure.

The *partition constant* is the number of cubic centimeters of 0.1 N alkali required to neutralize 25 cc. of the aqueous phase after 30 cc. of 0.1 N mixture of acids have been shaken with 20 cc. of the ether at 25°C.

The data for the use of the isoamyl ether-water system are presented because use of this system will be made in connection with the ethyl ether-water system in a method for the provisional identification of two fatty acids in a mixture. Although isoamyl ether would not ordinarily be used in routine determinations because of its relative cost, there is no reason, other than economy, for not doing so.

The mutual insolubility of isoamyl ether and water enhances its value and in this respect it is superior to the two ethers now employed in the partition method.

Isoamyl ether has a molecular weight of 158.2, a specific gravity of 0.774, and is insoluble in water but soluble in all proportions in ethyl alcohol or ethyl ether. It has a boiling point of 169-172° C. Upon vigorous shaking with water, it forms an emulsion which separates into insoluble phases slightly less quickly than the ethyl ether-water system.

The *partition constants* for the isoamyl ether-water system, given in table 1 were obtained at 25°C. by adjusting the original solution to 0.1N and partitioning the acids between 30 cc. of the 0.1N solution and 20 cc. of isoamyl ether. Twenty-five cc. of the aqueous phase were titrated with 0.1N alkali with phenophthalein as the indicator. Graphs of the partition constants as abscissae against percentage composition of the mixture are shown in figure 1.

Formic acid behaves similar to acetic acid and can not be determined accurately by the partition method. Fermentations do not generally produce significant quantities of formic acid and small quantities present will be calculated as acetic. Fermentations generally result in the production

TABLE 1. *Experimental values of isoamyl ether-water partition constants. Twenty-five cc. aqueous phase at 25 C.*

Percentage 0.1N acid first named	Acetic-propionic	Acetic-butyric	Acetic-lactic	Propionic-butyric	Propionic-lactic	Percentage 0.1N acid second named
100	24.3	24.3	24.3	21.4	21.4	0
90	24.0	23.6		21.0		10
80	23.75	22.90		20.5		20
70	23.5	22.10		20.1		30
60	23.2	21.40		19.6		40
50	22.9	20.60		19.2		50
40	22.6	19.90		18.7		60
30	22.3	19.19		18.2		70
20	22.0	18.40		17.8		80
10	21.7	17.6		17.4		90
0	21.4	16.9	26.0	16.9	26.0	100

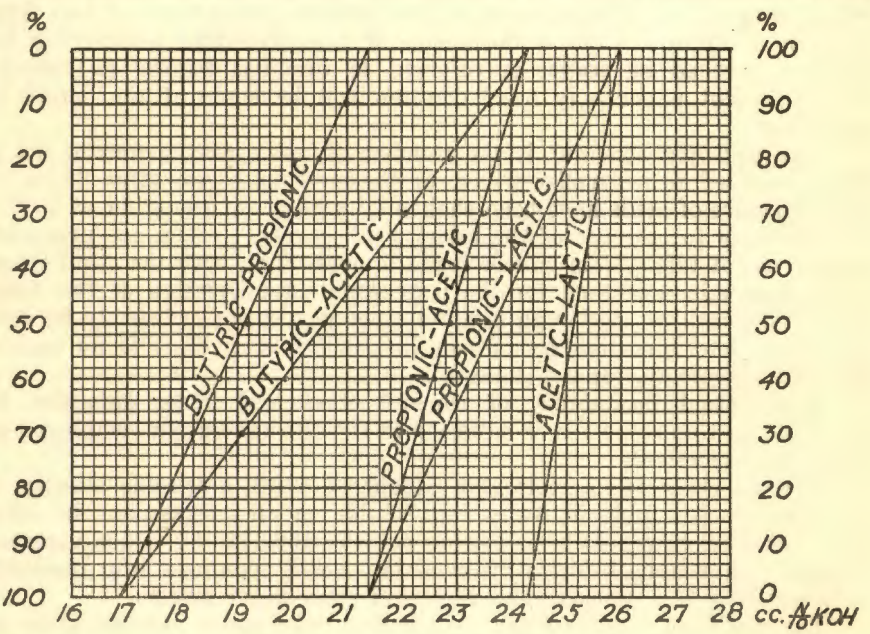

Fig. 1. Partition constants for the quantitative determination of two fatty acids distributed between isoamyl ether and water.

of substantial quantities of one or two volatile acids with small amounts of a third acid such as formic. The formic may be oxidized and thus determined if it is desired.

The general equations given in previous publications 1 and 2) for algebraic solution using the isopropyl ether and ethyl ether-water systems hold equally well for the use of isoamyl ether and will not be repeated here.

CONCLUSIONS

The partition method for the quantitative determination of two fatty acids in a mixture has been extended to include the use of the isoamyl ether-water system.

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HYDRATION IN SWEET CORN

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It is generally known that the seed of sweet corn often does not germinate as well as that of dent corn but the cause of this difference has not been determined. It seems, therefore, that a study of the intake of water under varying conditions might afford an explanation for the difference in germination of the two kinds of corn.

Babcock (2) found that when seed corn is placed in water, it will absorb more than its own weight in a few hours, and will increase considerably in volume. Duvel (6) stated that moisture is the prime factor causing the premature death of the seed.

Bailey (3) made a few preliminary tests upon the hygroscopicity of white dent corn and sweet corn. Fifty seeds of each were spread out on pieces of paraffined wire gauze which were suspended in the upper part of half-gallon museum jars, which contained different concentrations of sulfuric acid ranging from 30 to 85 per cent. The grain was exposed to the several atmospheres until no further change in weight took place. In all cases the dent corn absorbed a greater amount of moisture than the sweet corn.

Coleman and Fellows (4) studied the question of the hygroscopic moisture of cereal grains and flax, and demonstrated that absorption was greater for corn than for flax or wheat. Dillman (5) was interested in the hygroscopic moisture because of its bearing upon combine harvesting and subsequent storage. He pointed out that wheat, corn, buckwheat, and rice, possess about the same moisture content when equilibrium is reached.

METHODS OF SECURING DIFFERENT HUMIDITIES

The sweet corn employed in these studies was the well known variety, Stowell's Evergreen. The Reid's and Hogue's yellow dent varieties were employed for comparative tests. These ears were as nearly as possible of the same maturity as the sweet corn. The ears of corn were picked directly from the field, to represent four stages: (1) milk, (2) early dough or canning, (3) early dent, (4) mature. These were separated into lots, I, II, III, and IV. The corn was stored in a dry ventilated room where the temperature was approximately 22°C. Germination tests of both sweet and field corn showed that all seeds were viable. Before being used, all seeds were treated with hypochloride of lime.

To secure humidities of varying degrees, the sulfuric acid method of Wilson (8) was used. With a specific gravity of 1.05 there is 7.37 per cent acid, and a relative humidity of 97.5 per cent. For a humidity of 80.5 per cent, the amount of sulfuric acid is 27.32 per cent and the resulting specific gravity becomes 1.20. For a humidity of 60.7 per cent the concentration of sulfuric acid is 38.03 per cent, while the specific gravity has a value

of 1.29. The 100 per cent relative humidity was, of course, obtained by using distilled water alone. All of the hygroscopic tests were carried on in sealed containers. The sulfuric acid was held in a crystallizing dish resting upon the glass plate. Over the container was inserted an open topped bell jar. The rubber stopper of the bell jar served as a means by which the wire, holding the shallow screen tray with the seeds, could be suspended directly over the crystallizing dish 4 cms. from the water surface. A covering of brown paper excluded the light.

IMBIBITION OF SWEET CORN AND FIELD CORN

The purpose of the first series of experiments was to determine the extent or maximum absorption for sweet corn when left in distilled water. Ten-gram samples of sweet corn having a 9.1 per cent moisture content were placed in distilled water where they were allowed to remain for 72 hours: during this time they were weighed at frequent intervals. At the time of each weighing the water was poured off, and the adhering water absorbed with filter paper. The seeds were then returned to the original container and water added. The data secured are shown in table 1.

TABLE I. *Absorption in sweet corn seed submerged in distilled water*

Hrs. submerged	7	9	12	20	23	29	33	48	60	72
Percentage gain	29.6	32.9	38.8	53.2	57.1	62.7	66.3	76.7	87.1	96.9

At the end of 7 hours the average absorption was 29.6 per cent and after 72 hours 96.9 per cent. The rapid rate of absorption shortly after the seeds were placed in the water was particularly noticeable. It is clear that data should have been collected earlier.

The next series of experiments was planned so that particular consideration could be given to the extent of absorption for shorter periods of subjection than seven hours. Weighings were made at the end of 1, 2, and 4 hours, and extended over a longer period in order to be sure that maximum absorption had been reached. For a comparison, Houge's yellow dent corn was used. The data are given in table 2.

The data given in table 2 and the graph (Fig. 1) show that when sweet corn and field corn are placed in distilled water, the sweet corn absorbs a greater amount than dent corn. The equilibrium point for both the sweet corn and the dent corn is reached at about the same time. However, the sweet corn seed absorbs water to the extent of 113.37 per cent, while for dent corn the intake does not exceed 73.7 per cent.

THE RATE AND EXTENT OF ABSORPTION OF CORN SEED

It is desirable to determine the effect which various external factors may have upon the seed with varying moisture contents. An attempt was made to determine the rate and amount of absorption. The sweet corn used in these trials was the same variety but was different in moisture content; the Reid yellow dent has a water holding content of 8 per cent. The rate of absorption in distilled water is shown in table 3.

TABLE 2. *Percentage absorption occurring in Stowell's evergreen sweet corn and Hogue's yellow dent corn in distilled water for periods from one to 241 hours*

Time hours	Sweet corn	Dent corn
	Percentage gain	Percentage gain
1	11.85	8.87
2	17.37	13.97
4	26.15	20.30
7	33.35	25.55
9	39.34	29.20
11	43.07	32.25
13	46.97	34.25
15	49.30	38.42
20	55.22	39.85
22	58.82	41.27
24	61.40	42.99
28	65.15	43.07
32	67.52	46.77
41	74.92	50.67
45	78.20	51.27
50	80.25	53.37
54	82.22	54.62
62	86.82	57.97
74	92.40	60.42
87	97.45	64.87
100	101.72	66.55
112	105.15	69.10
124	107.86	70.35
148	110.75	72.12
172	113.32	73.70
241	113.37	70.45

TABLE 3. *Percentage increase in weight of sweet corn and dent corn in distilled water at 25°C.*

Sweet corn		Dent corn	
Percentage increase	Time-hours	Percentage increase	Time-hours
12 to 17	1 to 2	12 to 14	1 to 2
17 - 26	2 - 4	14 - 20	2 - 4
25 - 35	4 - 7	20 - 25	4 - 7
35 - 40	7 - 9	25 - 30	7 - 9
40 - 45	9 - 12	30 - 35	9 - 11
45 - 50	12 - 15	35 - 40	14 - 22
50 - 60	15 - 24	40 - 45	22 - 28
60 - 70	24 - 32	45 - 50	28 - 42
70 - 80	32 - 50	50 - 55	42 - 55
80 - 90	50 - 74	55 - 60	55 - 75
90 - 100	74 - 100	60 - 65	75 - 88
100 - 110	100 - 148	65 - 70	88 - 124
110 - 115	148 - 175	70 - 75	124 - 173

In the data given, the moisture content of the sweet corn having an initial value of 10 per cent produces an imbibitional increase of 40 per cent in nine and 22 hours respectively. At the end of 15 hours the sweet corn has taken up a sufficient amount of water to bring the increase in weight of the sweet corn up to 50 per cent, while the dent corn does not give this result until after a lapse of 28 hours. To produce an imbibition of 60 per cent the time factor was 24 hours for the first named strain and 55 hours for the second, while for 70 per cent, the respective subjection periods were respectively 32 and 124 hours. The maximum hydration was found to be approximately the same in both cases.

HYGROSCOPICITY OF SWEET CORN AND DENT CORN

Sweet corn and dent corn are often stored where the humidities are high. The next series of absorption tests was made to determine the differences in hygroscopicity between sweet corn and dent corn. The humidities arbitrarily chosen were 100, 95 and 90 per cent. The seed used in these tests was harvested at different stages of maturity and divided into four different lots: I Milk, II Canning, III Early dent, and IV Mature. The corn was dried and stored under identical conditions. The results secured in a saturated atmosphere are in table 4 and shown graphically in figure 2.

TABLE 4. *Absorption of moisture by sweet corn and dent corn in an atmosphere of 100 per cent humidity at 22°C.*

Lot	Variety of corn	Time in hours						
		20	44	68	94	116	140	168
I	Sweet	22.18	32.34	35.77	38.25	41.25	44.34	46.17
	Dent	17.65	27.82	32.60	34.15	37.99	42.37	43.97
II	Sweet	20.38	29.01	33.62	33.41	39.66	43.29	45.67
	Dent	15.41	24.17	32.92	.....	33.86	36.33	38.38
III	Sweet	15.98	26.38	34.36	41.08	47.08	51.73	54.78
	Dent	15.59	27.56	33.16	38.35	43.72	50.45	51.96
IV	Sweet	18.71	28.08	36.41	41.72	46.71	52.87	55.65
	Dent	18.74	33.22	42.01	46.55	51.34	56.49	48.41
Aver-age	Sweet	19.31	28.95	35.04	38.61	43.67	48.05	50.56
	Dent	16.84	28.19	35.17	29.74	41.72	46.41	45.68

Examining the data as submitted in table 4, it appears that lot I throughout the test shows a greater hygroscopicity for the sweet corn than for the dent corn; the same is true for lot II. In lot III the dent corn gives a higher reading. In lot IV the sweet corn has the lower value up to the 140 hour reading. Considering the average data the sweet corn is higher than the dent corn except on the 68 hour reading. When absorption by the less mature corn is compared with that of the more mature, there seems to be indications that the higher hygroscopicity is associated with maturity.

The next series of tests of hygroscopicity of corn was concerned with the extent of absorption in an atmosphere where the humidity was 95 per cent. The data secured are presented in table 5.

TABLE 5. *Rate of absorption of moisture by sweet corn and dent corn in an atmosphere of 95 per cent humidity at 22°C.*

Lot	Variety of corn	Time in Hours					
		20	68	94	116	140	168
I	Sweet	16.36	15.40	30.44	22.90	25.02	28.05
	Dent	14.23	15.73	23.14	19.93	26.99	23.06
II	Sweet	16.04	14.82	27.08	22.10	28.41	23.63
	Dent	12.20	14.59	22.20	18.27	25.10	24.32
III	Sweet	13.45	16.27	25.12	25.43	33.22	34.93
	Dent	12.74	15.39	24.23	22.78	32.02	30.31
IV	Sweet	16.20	19.85	27.33	26.32	34.62	31.14
	Dent	14.81	15.81	26.56	25.49	31.05	31.97
Aver-	Sweet	15.51	16.58	27.49	24.18	30.06	29.18
age	Dent	13.49	15.38	24.03	21.61	28.79	27.41

It is apparent that the resistance to absorption has been increased, for the maximum moisture intake was never above 30 per cent, while in the 100 per cent chamber the increase became almost twice as much. For the first 68 hours there is generally greater absorption registered for the sweet corn. Some time after an exposure of 68 hours there is a decided drop in the extent of the intake, for the 94 hour readings give a decrease, while 22 hours later there is a decided increase over the earlier readings. This apparent discrepancy may possibly be attributed to a drop in the room temperature. Maximum absorption is reached after an exposure of 116 hours; after that there is a decrease. This may be explained as being a result of germination.

The next series of hygroscopicity tests was made where the humidity was adjusted to 90 per cent. The method of procedure was the same as before. The data are presented in table 6.

TABLE 6. *Rate of absorption of moisture by sweet corn and dent corn in an atmosphere of 90 per cent humidity at 22° C.*

Lot	Variety of corn	Time in hous						
		20	44	68	94	116	140	168
I	Sweet	13.47	16.21	22.15	17.85	23.09	19.15	17.20
	Dent	13.02	14.45	17.89	18.38	25.75	20.48	18.16
II	Sweet	22.81	16.85	25.82	19.50	25.41	18.95	18.03
	Dent	13.69	14.68	21.76	18.27	23.70	18.75	18.09
III	Sweet	12.63	14.90	24.98	26.36	25.50	19.65	16.66
	Dent	13.21	15.33	24.71	26.42	34.14	29.50	26.30
IV	Sweet	20.80	18.12	28.76	24.30	30.97	26.55	22.91
	Dent	20.51	16.35	26.39	22.40	31.54	25.60	22.11
Aver-	Sweet	19.92	26.52	25.42	19.50	26.24	21.07	18.70
age	Dent	15.10	15.20	22.68	21.36	28.78	23.58	23.66

The results do not show correspondingly lower values between the 95 and 90 per cent humidity as noted between the 100 per cent and 95 per cent. With the lower humidity it is to be expected that the absorption would be somewhat less. This did not always prove to be the case. For the first 68 hours, however, the readings are consistently lower than those for the 95 per cent humidity, indicating an increased resistance to absorption. Lot IV (mature) for the first 116 hours shows a greater increase. It is noted also that there are lower values registered for 116 hours than for 94 hours or for 140 hours. The reason for this is probably the same as suggested for the lower readings at the 94th hour in the preceding experiment, namely a lowered temperature. It is noticed too, that the readings taken at the end of 140 hours are higher. The lower values recorded for the last readings are, with one exception, lower. Whether this can be attributed to increased respiration cannot be definitely stated.

In the data submitted previously there are indications that mature corn placed or held in an environment where the humidity is high will absorb a greater amount of moisture in a given time than cured and dry green corn kept in the same place. The data are given in table 7.

TABLE 7. *Percentage gain in sweet corn and dent corn of varying stages of maturity when subjected to a humidity of 100 per cent for varying periods from 20 to 168 hours*

Lot	Variety of corn	Time in hours						
		20	44	68	94	116	140	168
I	Sweet	19.02	26.00	27.47	30.28	31.05	33.10	34.61
	Dent	16.98	23.16	21.86	27.25	30.32	33.58	32.26
II	Sweet	17.08	22.94	27.90	27.35	30.22	32.37	32.42
	Dent	16.11	22.06	24.96	24.36	28.11	30.40	31.60
III	Sweet	16.35	23.82	27.33	31.86	36.65	40.88	42.38
	Dent	15.31	30.10	25.41	31.80	36.07	40.39	41.23
IV	Sweet	24.77	21.94	29.68	34.29	38.48	43.41	44.45
	Dent	20.30	38.87	32.59	35.60	40.81	44.08	43.33

The data as given in table 8 point toward a gradual increase in hydration capacity with maturity. It is true that there is little difference in the results submitted for lots I and II. This is not unexpected, for the time of transition is exceedingly short and somewhat indefinitely marked, (1). The corn selected from lots III shows a much higher hygroscopicity and the same is true for the corn of lot IV. The differences in hygroscopicity represented by sweet corn and dent corn for lots or stages I, II, and III are shown in graphs in figures 3, 4 and 5. Probably the increased hydration of mature sweet corn and dent corn over the immature is caused by the production of pentosans (7), and other materials which increase the capacity for water intake.

If corn has a lower hygroscopicity when harvested and cured during the early dented stage, it is logical to advocate the harvesting of seed corn at such a time.

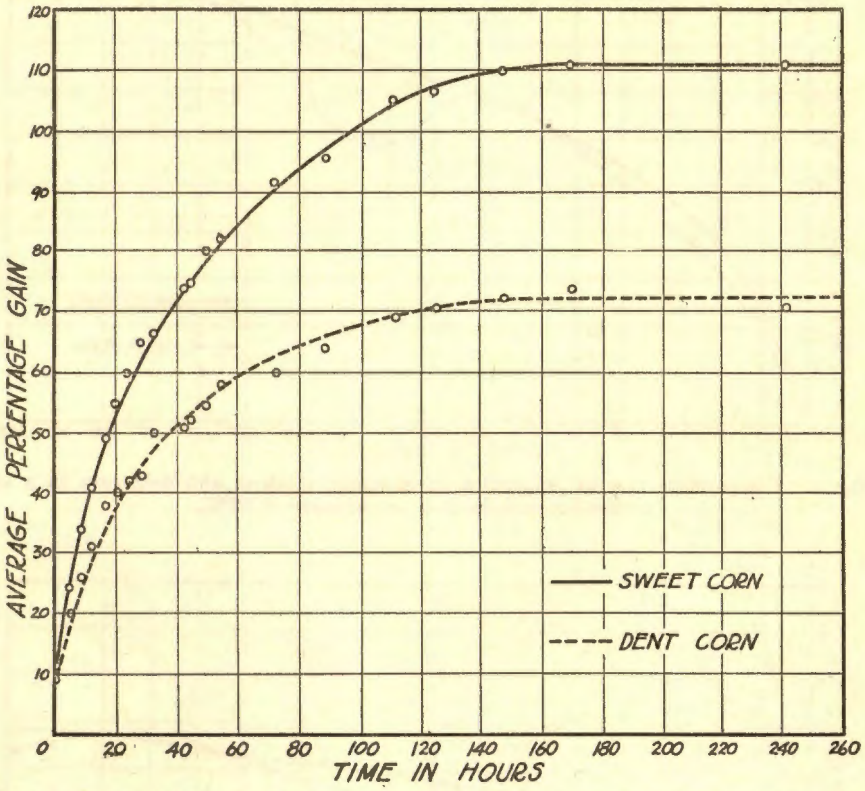

Fig. 1. Comparative rate of absorption of moisture in sweet and field corn. Note that the sweet corn absorbs considerably more moisture than the field corn in the same period of time.

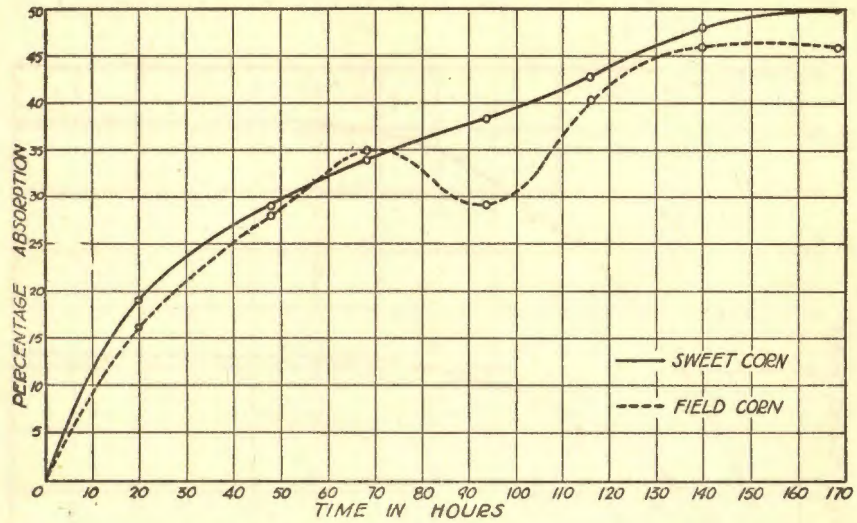

Fig. 2. Comparative rate of absorption of moisture of sweet and field corn in a saturated atmosphere at a temperature of 22°C.

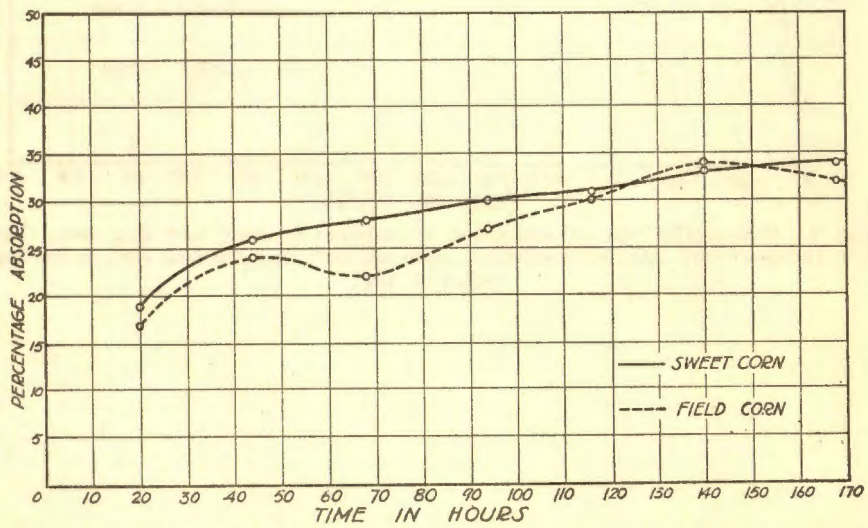

Fig. 3. Comparative rate of absorption in sweet and field corn which were picked in the milk stage, dried and then subjected to a saturated atmosphere.

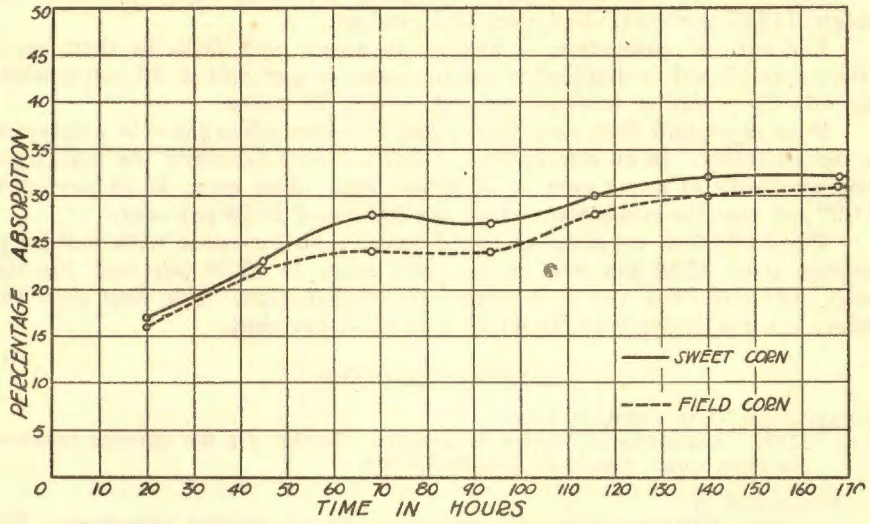

Fig. 4. Comparative rate of absorption in sweet and field corn which were picked in the canning stage, dried and then subjected to a saturated atmosphere.

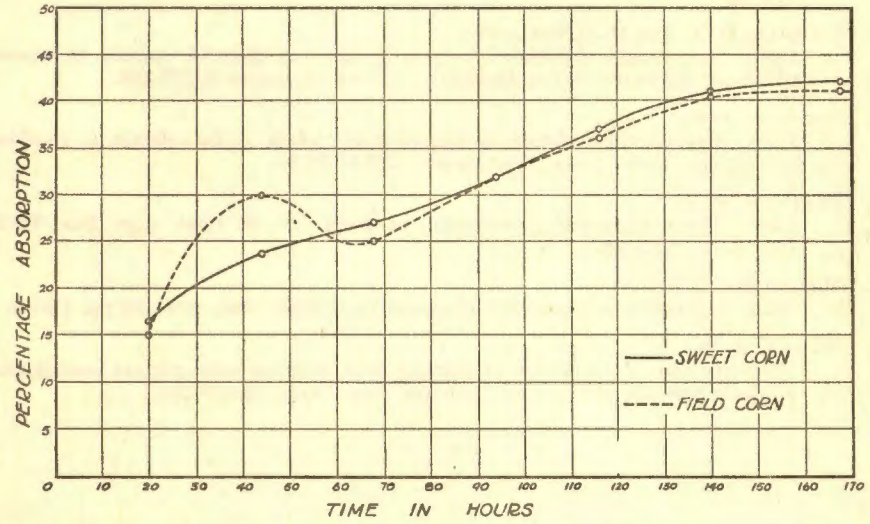

Fig. 5. Comparative rate of absorption in sweet and field corn which were picked in the early dent stage, dried, and then subjected to a saturated atmosphere.

SUMMARY

Cured sweet corn seed when placed in distilled water increases its weight 113.37 per cent; dent corn 73.7 per cent.

The rate of absorption is greater in sweet corn than in dent corn. Sweet corn placed in distilled water increases 50 per cent in 15 hours while the rate for a similar increase in dent corn is 28 hours.

Both sweet and dent corn absorb less moisture when there is a decrease in the humidity. In an atmosphere of 100 per cent humidity the maximum hygroscopicity of sweet corn is 50.56 per cent; dent corn, 45.68 per cent. At 90 per cent the respective values are 30.06 and 28.79 per cent.

The hydration capacity of cured sweet corn increases with maturity, passing from 32.42 per cent in the milk stage to 42.38 per cent for the early dent and 44.45 per cent when harvested mature. For dent corn the values are respectively 31.60, 41.23 and 43.33 per cent.

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THE CANNIZZARO REACTION WITH FURFURAL[1]

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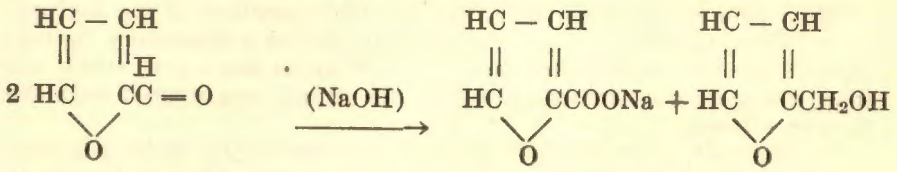
INTRODUCTION

The Cannizzaro reaction is one of the best methods for the preparation of acids and alcohols from aldehydes. It has been extensively used for the synthesis of pyromucic acid and furfuryl alcohol from furfural[2].



The need for improvements in this reaction has become urgent in view of the increasing number of substituted furfurals developed in this laboratory[3].

As a result of a miscellany of studies reported at this time, it is now possible to carry out the Cannizzaro reaction on furfural with greater convenience and improvements in yield. The method, which involves the use of an alcoholic solution of sodium hydroxide, is recommended particularly for substituted furfurals which do not contain a substituent sensitive to alcoholic-alkali solutions. It should also prove suitable for small-scale laboratory condensations involving furfural. Actually, the method has been tried with success as a student laboratory exercise in an undergraduate course in organic chemistry[4].

Hill[5] used alcoholic-sodium hydroxide and Hartley and Dobbie[6] used alcoholic-potassium hydroxide to prepare pure pyromucic acid from fur-

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[1]This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge help from the Industrial Science Research Fund for the partial defrayal of expenses incurred in this investigation.

[2]Leading references to this reaction may be found in Bulletin No. 2, June, 1928, entitled "Furfural and Its Derivatives," published by the Miner Laboratories, Chicago, Ill. See, also, *Organic Syntheses*, 6, 44-47 (1926).

[3]See, Gilman and Wright, *J. Am. Chem. Soc.*, 52, 1170, 2550 (1930), for the preparation of bromofurfural and nitrofurfural. Since then other new substituted furfurals have been synthesized in this laboratory.

[4]Recently Gilman, Brown, Wright and Hewlett, *Iowa State College Jour. Sci.* 4, 355 (1930), directed attention to the Perkin reaction with furfural (leading to the preparation of furylacrylic acid) as another student preparation. Such preparations are admirably suited for general laboratory instruction in organic chemistry, and have the special merit of starting with the very inexpensive furfural.

[5]Hill, *Am. Chem. J.*, 3, 33(1887).

[6]Hartley and Dobbie, *J. Chem. Soc.*, 73, 598 (1898).

fural. They made no study of optimal conditions, did not isolate the furfuryl alcohol, and did not report yields of the pyromucic acid. Their alkali was dissolved in ethyl alcohol. In our experiments, only methyl alcohol was used, partly because we were interested in a preparation for undergraduates which would not involve the use of ethyl alcohol. For technical purposes, the use of our sodium hydroxide is, of course, to be preferred to potassium hydroxide.

Studies are in progress on the interconversion of the so-called water soluble and water insoluble forms of furfuryl alcohol. If it should prove possible to readily effect such interconversion then the isolation of alcohol from the Cannizzaro reaction should be markedly simplified.

EXPERIMENTAL PART

Three different methods were used for the extraction of the furfuryl alcohol. First, the reaction mixture was extracted in a separatory funnel; second, extraction was effected in a Soxhlet apparatus; and, third, the sodium pyromucate contained on a Büchner funnel was washed with the extracting solvent.

The furfural was redistilled material, and the sodium hydroxide solution was prepared by dissolving 20 g. (0.5 mole) of sodium hydroxide in 145 cc. of methyl alcohol. Such solution was accelerated by stirring or shaking.

In the first series of experiments (extraction in a separatory funnel), 96 g. (1.0 mole) of furfural was mixed with the solution containing 20 g. (0.5 mole) of sodium hydroxide in 145 cc. of methyl alcohol. The reaction was carried out in a Erlenmeyer flask. A yellow mass precipitated in a very short time and set to a solid with a reddish tint. The reaction is distinctly exothermic, and for this reason cooling was effected by tap water. After standing (generally for twelve hours) the mixture was dissolved in water and extracted with ether until the extracts were only slightly red in color. The ether extracts were distilled at atmospheric pressure and that portion distilling between 167°-170° was collected separately. This was the furfuryl alcohol, and its pale yellow color changed on standing to a reddish color[7]. The solution containing sodium pyromucate was heated on a water-bath to expel residual ether, and was then acidified with 50 per cent sulfuric acid. In this manner, the yellowish crystals of pyromucic acid (melting at 127°) were precipitated. Experimental details of this method are contained in table 1, which describes a series of preparations starting with one mole of furfural and one-half mole of sodium hydroxide.

Two experiments were carried out using a Soxhlet extractor. Each mixture resulting from 0.5 mole of furfural and 0.25 mole of sodium hydroxide was allowed to stand 12 hours prior to transferral to a Soxhlet extractor. Extraction with 250 cc. of ether was continued until the ether washings were clear. The extracts were distilled in the customary manner, again collecting the furfuryl alcohol between 167°-170°. The sodium salt

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[7]The use of a very small amount of urea has been recommended as a stabilizer for furfuryl alcohol. (See, *Organic Syntheses*, **6**, 46 (1926).

TABLE I. *Extraction of furfuryl alcohol in separatory funnel*

Solvent used in extraction	Vol. of solvent	Pctg. yield of acid	Pctg. yield of alcohol
Ether	800 cc.	58.0	87.0[a]
Ether	800 cc.	30.0	33.6[b]
Ether	200 cc.	25.0	27.0[c]
Benzene	800 cc.	56.0	42.0[a]
Benzene	200 cc.	20.0	35.0[d]
Carbon Tetrachloride	200 cc.	.....	10.8[e]

[a] The condensation mixture was allowed to stand at room temperature 12 hours before being worked up. It remains for further experiments to determine whether this time for reaction can be materially decreased.

[b] In this experiment the mixture was kept below 50°, and the time of standing was 5 hours.

[c] Very probably the 200 cc. of ether used for extraction in this experiment was inadequate, because on acidification of the sodium salt a tarry mass developed. This indicates the presence of unextracted furfuryl alcohol because the pyromucic acid is stable towards the inorganic hydrolyzing acid under these conditions.

[d] Here, also, the formation of an apparently intractable tarry mass on acidification indicated that not all of the furfuryl alcohol had been extracted.

[e] The acid was not recovered from the tarry mixture resulting on acidification.

Attention should be directed to a single experiment where extraction was replaced by steam distillation. Only a small quantity of furfuryl alcohol was obtained in the distillate, and when the residue from steam distillation was acidified a thick, gummy mass formed, and this yielded 18 per cent of acid.

was acidified with 50 per cent sulfuric acid, and the acid obtained in this manner melted at 127°. In an 8 hour period of extraction, the yield of acid was 70 per cent and the yield of alcohol, 50 per cent. In a corresponding experiment where the time of extraction was but one hour, the respective yields were essentially the same.

In the third group of experiments, the reaction mixture (after standing for 12 hours) was transferred to a Büchner funnel and there washed with ether until the washings were essentially free of color. The furfuryl alcohol and pyromucic acid were then worked up in accordance with the procedures described for the other two methods. The boiling point and melting point, respectively, of the two products were alike in all three methods. Two experiments were carried out under these conditions with 1.0 mole of furfural and 0.5 mole of sodium hydroxide. The yields were: 50 per cent and 75 per cent, respectively, of acid; and 56 per cent and 34 per cent, respectively, of alcohol. In that experiment giving a 50 per cent yield of acid, some of the sodium salt of the acid was lost. Also, two experiments were carried out using 3.0 moles of furfural and 1.5 moles of sodium hydroxide. Here, the yields of acid were 71 per cent and 75 per cent, respectively; and the yields of alcohol, 41 per cent and 49 per cent, respectively. In these 3.0 mole runs it is necessary to cool the mixture in an ice-water bath to about 0°, otherwise a too vigorous reaction occurs.

It is evident that each of the three methods described has special advantages. The choice of a particular method for extracting the furfuryl alcohol depends somewhat on the size of a preparation and the availability of the substituted furfural used. Obviously, with a rare substituted-furfural it would be desirable to use that method which gives the highest yields of acid and of alcohol. It should be remembered that a large part of the solvent used for extraction can be recovered by usual procedures.

The authors gratefully acknowledge assistance from Mr. George F. Wright.

SUMMARY

Conditions have been described for effecting a Cannizzaro reaction with furfural and sodium hydroxide dissolved in methyl alcohol. The method is recommended particularly for substituted furfurals because of the improved yields and general convenience over other related procedures.

A POLYMERIC 2-FURFURYL MERCAPTAN[1]

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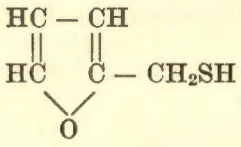
From the Chemical Laboratory of Iowa State College

Accepted for publication July 15, 1930

INTRODUCTION

In connection with studies[2] concerned with applications of 2-furfuryl mercaptan, a compound obtained directly from furfural, it was noted that the exceedingly offensive smelling liquid mercaptan underwent a change on standing in a sealed tube to give an odorless solid. This metamorphosis is apparently not described in the literature. In order to elucidate the structure of the solid, it was distilled under reduced pressure and a chief component of such distillation was the liquid mercaptan. The solid and the liquid have the same percentage of sulphur and this with the interconversion of one form to the other is evidence that the solid is a polymer of the liquid 2-furfuryl mercaptan, the structural formula of which is



In this polymeric modification of 2-furfuryl mercaptan, we have one of the most striking illustrations of an all too frequently occurring phenomenon of furan compounds: namely, their tendency to ready, and in some cases complete, conversion to oils, tars and resins. The formation of such apparently intractable materials is the *bête noire* of furfural chemistry, because of the depressing ease with which many furan compounds assume almost intractable modifications. At the same time we should hasten to add that this same tendency to resin formation is, of course, of fundamental importance in the technical preparation of resins, plastics, and lacquers, from furfural and its derivatives.

It is well-known that there exists wide differences in the so-called stabilities of furan types. Some, like the acids, are uncommonly stable; others, like furan itself, amino furans and furan alcohols, are very unstable. Also, recent studies[3] have demonstrated that it is possible to pronouncedly stabilize otherwise labile molecules by the introduction of vari-

[1]This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge assistance from the Industrial Science Research Fund for the partial defrayal of expenses incurred in this investigation.

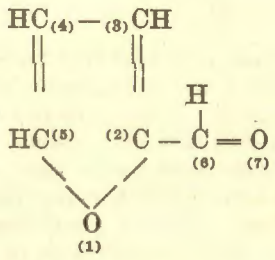
[2]Gilman and Hewlett, *J. Am. Chem. Soc.*, 52, 2141 (1930).

[3]A series of such studies has been reported from the laboratories of organic chemistry in the last few years in the *Iowa State College Jour. Sci.*, in the *J. Am. Chem. Soc.*, and in the *Rec. trav. chim.*

ous substituents (like the nitro-group) and by the so-called protection of groups (the formation of diacetates, etc., of the aldehyde group, and ethers and esters of the alcohol group, etc.).

There is not at present any single and conclusive explanation for the alterations which furan compounds undergo on standing, by the application of various physical processes like heat, light and pressure, and by virtue of the presence in varying quantities of a miscellany of catalysts[4]. However, we are of the opinion that such changes are very largely inherent in the peculiar arrangement of atoms which goes to make up the furan nucleus. We are led to this view by two observations. First, the definite illustration reported at this time of the polymerization of 2-furfuryl mercaptan is probably a phenomenon exclusive of the mercapto (-SH) group, because of the relative stability of compounds having this grouping, when the compounds are not subjected to atmospheric oxidation. Second, the unusual variety of furan compounds which undergoes alteration to complex forms indicates that it is a property inherent in the furan nucleus, not excepting, however, contributions to such action offered by substituents—and particularly those substituents having a real or latent unsaturation.

If it is true that the seat of such transformations, and we shall designate them somewhat loosely and empirically as polymerization phenomena, is in the furan nucleus then it is desirable to inquire as to the groups of this nucleus which might participate in the changes. There are various types of polymers and a number of explanations for their formation[5]. With furan compounds, such polymerization can arise in a number of ways. Selecting furfural as a type



and numbering the several key elements in the indicated manner we are at once struck with the several conjugated systems. These are: 2, 3, 4, 5; 3, 2, 6, 7, and, if the nuclear oxygen is designated as an unsaturated element capable of functioning in an oxonium form, then we have two additional conjugated systems; 1, 2, 3, and 1, 5, 4.

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[4]A report will appear soon on the effect of so-called polymerizing agents on furfural and its derivatives. This work is being done by N. O. Calloway.

The transformations which furan compounds undergo may be effected in many cases by a sort of auto-catalysis, the catalyst arising from the incipient decomposition or transformation of supposedly pure compound. Also, it must be remembered that the walls of containing vessels (with or without absorbed gases or moisture or reactants) can effect changes on compounds contained therein.

In a number of cases it has been observed that moderately low temperatures have a pronounced stabilizing effect on furan compounds.

[5]See current literature for the studies by Staudinger, Carothers and others.

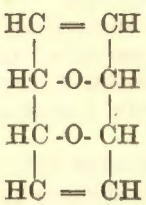
We should emphasize that a large number of furan types may undergo profound changes not only by polymerization, but also by intramolecular and intermolecular condensations and rearrangements which may be preceded or accompanied by ring scission.

In addition to the highly reactive conjugated systems just mentioned there are the simpler units of unsaturation: 2, 3, and 4, 5, in addition to the nuclear oxygen and the extra-nuclear carbonyl group 6, 7.

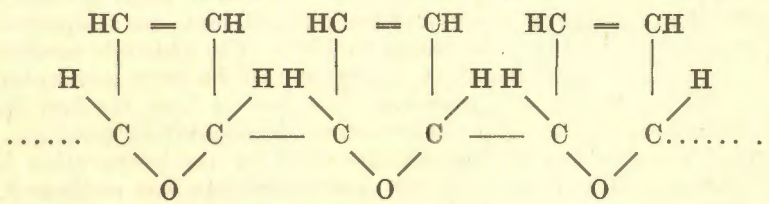
Finally, there is another possible highly reactive group which may participate in polymerization, and that is the enol (or the di-enol, in some cases) resulting from ring scission. It appears that the furan ring can be opened with relative ease by a number of reagents, and it is definitely known that the enol grouping is one of the most active organic linkages.

We have then at least three distinct types of likages, real and potential, in furan compounds, any one or any combination of which might be responsible for the union of two or more molecules to give polymers. For want of any definite experimental evidence at this time, it is not possible to designate any one or any combination of such linkages which participate in all polymerizations. When polymerization involves extra-nuclear linkages, like the carbonyl group in furfural, then we have a peculiar system for each furan compound. The same is true, but to a lesser extent, in those cases where only the furan ring or better stated where essentially the furan nucleus takes part. In this latter case it is now possible to have a picture of the configuration or of the mode of linking of some polymers.

If we confine attention, for purposes of illustration, to the 2, 3, 4, 5, conjugated system[6], then by 1, 4-addition of two molecules of furan itself we would have the following polynuclear heterocyclic type



If, however, we use the same compound and the same conjugated system, but have the polymerization extend beyond two units, then the following is a picture of such polymers:



The partial or latent or unsatisfied linkages designated by dotted lines can then be starting points for the addition of further units or for completing a system intramolecularly[7].

[6]This system is selected because it is one of the chief systems designated by Gilman and Wright, *J. Am. Chem. Soc.*, **52**, 3349 (1930), in an explanation of some substitution reactions of furfural.

[7]It should be noted that in these particular forms that a new unit of activity has been developed: namely, the unsaturation between elements 3 and 4. Should this unit prove to be relatively more reactive than the others in the designated molecules then it might prove possible to synthesize furan types with substituents on elements 3 and 4. Such elements otherwise greatly resist replacement of their hydrogen by substituents.

It should be possible to determine the correctness of the polymers indicated (as well as the other types which may be constructed from the other linkages mentioned) by the physical and chemical methods now available and in the process of being developed for other studies on polymerization. The problem is one of fundamental importance in extending our knowledge of furan compounds, particularly because of the apparent ease with which so many furan types polymerize[5].

In some cases (see footnote 7) it may prove highly desirable to effect such polymerization and then depolymerize the molecule. This brings to mind the so-called water-insoluble form of furfuryl alcohol, $C_4H_3OCH_2OH$. The structure of this form is unknown. It may or may not be a polymer of the simple furfuryl alcohol, that remains to be determined from some studies now in progress. It is significant that the furfuryl alcohol is a simple analogue of thio-furfuryl alcohol or 2-furfuryl mercaptan. Should the water-insoluble form of furfuryl alcohol prove to be a polymer which would be readily converted to the water-soluble furfuryl alcohol, then it would be a ready means of decidedly improving the yield of furfuryl alcohol in the Cannizzaro reaction[8].

EXPERIMENTAL PART

The 2-furfuryl mercaptan[2] was sealed in a glass tube and allowed to stand for one year. At the end of this time, the contents had become highly viscous and a white solid was suspended in the mixture. Filtration by suction served to remove the viscous oil from the solid. The oil had the highly characteristic odor of furfuryl mercaptan, but resisted steam distillation and underwent complete decomposition upon attempted distillation under reduced pressure.

The solid was thoroughly washed with anhydrous ether and then crystallized from ethyl acetate to yield an apparently pure compound, without odor, and melting sharply at 135°.

Analysis Calc. for $(C_5H_6OS)_n$: S,28.07%. Found: S,27.98%.

The value of n has not as yet been determined with definiteness, but it may be as high as 7 by boiling point determinations in ethyl acetate[9].

Three grams of the polymer were heated at 10 mm., the temperature of the oil-bath being gradually increased to 150°. The distillate consisted of one gram (a 33.3% yield based on a polymer) of furfuryl mercaptan distilling at 155° at atmospheric pressure. The residue from the first distillation appeared to consist of a carbonaceous decomposition product. The furfuryl mercaptan was further characterized by the preparation of the *p*-nitrobenzoate. The identity of the *p*-nitrobenzoate was confirmed by a mixed melting point determination with an authentic specimen.

In a miscellany of studies by J. B. Dickey, G. F. Wright, R. E. Brown, E. A. Zoellner, S. A. Harris, W. M. Selby, N. O. Calloway and others in this laboratory it has been observed that furan compounds, as might have

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[8]See Gilman and Selby, *Iowa State College Jour. Sci.* (1930), for the Cannizzaro reaction. One of the major difficulties in this excellent synthesis is the removal or recovery of furfuryl alcohol.

[9]It is interesting to recall that molecular weight determinations of furan compounds indicate a general tendency to association.

been expected, show a greater tendency to remain pure when first purified with care and then carefully sealed[10]. With some liquids it appears desirable to effect such purification by distillation *in vacuo* rather than at atmospheric pressure.

A number of compounds which have been kept for varying times appear to be undergoing transformations which might be like that of furfuryl mercaptan. An interesting illustration is the simple ethyl furoate which is gradually being converted to a higher boiling compound.

It is noteworthy that some of the pure, freshly distilled furan compounds only develop their highly characteristic odors (and these are very pleasant with some esters, etc.) about one-half to one hour subsequent to distillation.

A study now in progress on "stabilizers" for furan compounds should make it possible to extend the utility of furan types.

SUMMARY

It has been shown that the liquid 2-furfuryl mercaptan polymerizes spontaneously to a solid, and that the polymer so formed can be converted by heating to the liquid. The solid form has none of the extremely obnoxioux odor so characteristic of the liquid. In this preliminary study a series of mechanisms has been postulated for polymerizations of furan types, and some applications of the polymers have been considered.

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[10]There are some cases where it appears to be of advantage not to seal the compounds in order to permit volatile products of decomposition to escape and so diminish auto-catalytic decomposition or alteration.

It is interesting to note that furfural, quite unlike the analogous benzaldehyde, greatly resists atmospheric oxidation to furoic acid under conditions where benzaldehyde is readily converted to benzoic acid. However, preliminary experiments on the effect of ultra violet on furan compounds indicates a reaction between furfural and air at room temperature and in sealed containers, as a result of which the volume of confined air is reduced.

THE VARIETAL RESPONSE AND INHERITANCE OF RESISTANCE IN BARLEY TO ERYSIPHE GRAMINIS HORDEI P. F. 4

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From the Department of Botany, Iowa State College

Accepted for publication July 18, 1930

Erysiphe graminis hordei Marchal (powdery mildew) is a destructive parasite in most sections of the United States where barley is grown. Observations have shown that the conditions which favor the development of this parasite are cool temperature and humid atmosphere. However, the losses are particularly severe in the southern United States where barley is planted in the fall. The eastern half of the Mississippi Valley and coastal states in the western United States suffer an annual loss. In Iowa the barley has been relatively free from powdery mildew during the past twelve years, while in the neighboring states it has been severely attacked. The reason for this is not known.

At present there is available only limited information of the varietal response and the inheritance of resistance to *E. graminis hordei*. In 1907 Biffen [2] showed that resistance to *E. graminis hordei* was an inheritable character in crosses of *Hordeum spontaneum* (resistant) on *H. hexasticofurcatum* (susceptible). It is improbable, however, that he worked with a single physiologic form, because his experiments were carried out under field conditions on mature plants. The F_2 segregated into 56 susceptible, 16 intermediate, to 7 free plants. He reports no F_3 progeny tests.

Recently Mains and Dietz[3] isolated five physiologic forms of *E. graminis hordei*. These were distinguished by the response of four varieties of barley as follows:

Nepal C. I. 595 resistant (type 1-2)	p.f. 1
Nepal C. I. 595 susceptible (type 4)	
Peruvian C. I. 935 susceptible (type 3-4)	
Goldfoil C. I. 935 susceptible (type 4)	p.f. 5
Goldfoil C. I. 928 resistant (type 0)	p.f. 3
Peruvian C. I. 935 resistant (type 0-1)	
Blackhull-less C. I. 666 resistant (type 1-2)	p.f. 2
Blackhull-less C. I. 666 susceptible (type 4)	p.f. 4

Although the discovery of physiologic specialization complicates the production of a resistant variety of barley by hybridization, such an attempt is being made because of the economic importance of barley mildew. This paper deals with the varietal response and inheritance of resistance to a single physiologic form.

[1]The writer wishes to gratefully acknowledge the assistance of Dr. I. E. Melhus in the preparation of this manuscript.

[2]Biffen, R. H., 1907, Jour. Agr. Sci., 2:109-129.

[3]Mains, E. B., and S. M. Dietz, 1930, Phytopathology 20:229-239.

MATERIALS AND METHODS

Of the 90 varieties of barley whose reactions to mildew are reported in this paper, 88 varieties were secured from E. B. Mains, Purdue Agricultural Experiment Station, and two from Charles Marsh of Minnesota. The five varieties of barley used as parents of hybrids, together with their response to *Erysiphe graminis hordei* p.f. 4, are as follows: Chevalier C. I. 156 (susceptible, type 4), Goldfoil C. I. 928 (resistant, type 0), Odessa C. I. 927 (susceptible, type 4), Triebi C. I. 936 (susceptible, type 4) and Velvet C. I. 4252 (susceptible, type 4).

Physiologic form 4 was isolated from varieties of barley that served as differential hosts. This form was collected in the field at Moscow, Idaho, by the writer in 1926, by J. Milford Raeder in 1927, and at Davis, California, by Lysle D. Leach in 1927, 1928 and 1929. At the present time it is known to occur only in western United States.

The following hybrids and their reciprocals were made in the greenhouse at Ames, Iowa, during the winter of 1926: Chevalier C. I. 156 x Goldfoil C. I. 928; Triebi C. I. 936 x Goldfoil C. I. 928; Odessa C. I. 927 x Goldfoil C. I. 928, and Goldfoil C. I. 928 x Velvet C. I. 4252. The 22 F_1 plants were not exposed to infection with powdery mildew and were matured in the field during 1927. During the spring of 1928, each individual F_1 seed was planted in a three-inch pot in the greenhouse. The resulting plants were transplanted to the field after being exposed to infection with powdery mildew and their reaction noted. During the spring of 1929, the F_3 progeny tests were made in the greenhouse by planting the seed from each F_2 plant in separate rows two inches apart.

When the seedling hybrids had produced their second and third leaves, they were exposed to infection by atomizing with water and shaking heavily mildewed plants over them. These seedling plants were incubated over night—a period of twelve hours—in a moist chamber at 60-65° F. with a relative humidity of 85-90. The plants exposed to infection remained in the opened chamber until 4 p. m. of the same day, when they were removed to the greenhouse bench.

Th reaction of the hybrids was recorded when the powdery mildew had reached its maximum development, this being usually in 8 to 12 days. In order to avoid classifying escaped plants as resistant, all plants recorded as resistant were again exposed to infection.

The types of infection were divided into five arbitrary classes of host reaction. Three of the classes, designated by the symbols 0, 1 and 2, were considered resistant, and two, symbols 3 and 4, were considered susceptible. The five classes and types of infection are shown in table 1.

The parents used in crossing were sharply contrasted, the resistant plant was read as type 0, and the susceptible one, type 4. The hybrids, too, were sharply contrased, those classed as resistant were read types 0 to 1, and susceptible, types 3+ to 4.

THE RESPONSE OF VARIETIES OF BARLEY TO ERYSIPHE GRAMINIS HORDEI P. F. 4

Three plantings of the 90 pure line varieties of barley were each exposed to infection with *Erysiphe graminis hordei* p.f. 4. Classifying resistance as types 0 to 2 and susceptibility as types 3 to 4, 62 varieties were susceptible and 28 resistant (table 2).

TABLE 1. *Classes of reaction of barley to powdery mildew, Erysiphe graminis hordei*

Symbol	Classes of host reaction	Types of infection
0	Highly resistant	Macroscopically, no mildew. Chlorotic or necrotic flecks formed in some varieties. Microscopically sometimes a slight development of mycelia and haustoria.
1	Very resistant	Slight development of mycelia with little or no sporulation. Chlorotic or necrotic areas in some varieties.
2	Moderately resistant	Moderate to abundant development of mycelia with slight sporulation. Chlorotic or necrotic areas in some varieties.
3	Moderately susceptible	Moderate to abundant development of mycelia with moderate sporulation.
4	Very susceptible	Abundant development of mycelia and abundant sporulation.

Goldfoil C. I. 928, Unnamed C. I. 96, Hanna C. I. 906 and Duplex C. I. 2433 are particularly intereting as they gave an 0 type of reaction. The following good commercial varieties are completely susceptible: Horsford C. I. 610, Chevalier C. I. 278, Hanchen C. I. 531, Manchuria C. I. 245, Nepal C. I. 475, Odessa C. I. 927, Oderbrucker C. I. 940, Triebi C. I. 936 and Velvet C. I. 4252.

INHERITANCE OF RESISTANCE

Erysiphe graminis hordei is a limiting factor in many sections of the United States. Apparently there is considerably less damage in Iowa, however, than in adjoining states. It would be highly desirable to produce varieties of barley resistant to all physiologic forms, but first it is necessary to make a more comprehensive survey for physiologic forms in addition to those known, and further, to ascertain their geographic distribution.

As stated earlier, the F_1 hybrids were not exposed to infection, since a severe reduction in yield might result if susceptibility were dominant.

MILDEW REACTION OF THE F_2 PLANTS

Seven hundred ninety F_2 plants were secured from the 22 F_1 plants, the latter being the product of four different parental combinations. Of the total number of F_2 plants, 588 were susceptible to *E. graminis hordei* p.f. 4 and 202 resistant (table 3). Interpreting this on the basis of a 3:1 ratio, the calculated number of susceptible plants would be 592.5 and resistant 197.5. This leaves a deviation of 4.5 plants, between the observed and calculated results. The F_2 ratio of each cross approximates a 3:1 ratio, although the numbers in some cases are small.

The largest number of F_2 plants from a single cross was derived from Odessa x Goldfoil. A total of 554 F_2 plants was secured; 421 were susceptible and 133 were resistant. On the basis of a 3:1 ratio, a deviation of 5.5 plants between the observed and calculated numbers occurred.

The reaction of all F_2 plants to physiologic form 4 was so definite that only one F_2 plant was misclassified on the basis of the F_3 progeny test.

The absence of intermediate types of reaction allowed an easy classification of the hybrid response.

The foregoing results strongly indicate that susceptibility to *Erysiphe graminis hordei* p.f. 4 is dominant, and is caused by a single pair of factors. This indication is further verified by the F_3 progeny tests.

TABLE 2. *Reaction of barley varieties to Erysiphe graminis hordei p.f. 4*

Variety	No. C. I.	Reaction
Abyssinia	362	2
Abyssinia	1234	2
Arequipa	1256	2
Arlington Awnless	702	1
Barquis	1076	3
Blackhull	596	4
"	878	1
Blackhull-less	666	3
"	1032	4
"	1097	4
Bohemian	204	4
Bolivia		4
"	1257	2-3
Cabeza	1437	2
Chevalier	156	4
"	278	4
Chile Common	663	1-2
Chilean C.	1432	2
Chilean D.	1433	2
Club Mariout	261	3-4
Coast	690	2
Consul	1061	3
Country Barley	276	2
Czech	1023	3
Duplex	2433	0
Eagle	913	4
Evans	621	3
Featherston	911	4
"	1120	4
Gatami	575	4
"	1413	4
Gehangir	1089	3
Goldfoil	928	0
Hanchen	531	4
Hanna	30	3
"	203	4
"	906	0
"	942	4
Hansee Hull-less	703	1
Horsford	507	4
"	610	4
Heil Hanna 1.	681	4
Heil Hanna 3.	682	3-4
Himalaya	254	4
"	620	4
Hisein	1053	3
Hooded Spring	716	4
Horn	866	4
Huwan	1080	3
Juliaca	1114	4
Kimberly	1382	2
Kwan	1016	3
Leh	700	3
Lihor	866	4
Luth	922	1
Lynch	119	1
Manchuria	245	4
Nepal	475	4
"	489	4
"	595	4
"	598	4
"	724	3
"	1290	4
"	1292	4
Nigrate	2444	1
Oderbrucker	940	4
"	969	4
Odessa	182	3
"	927	4
"	961	4
Oswong	697	2
Palestine	939	1
Pannier	1330	4
Peru	653	3
Peruvian	935	1
"	1131	1
Poda	652	3
Pontius	731	3
Princess	603	4
Purple Nepal	1373	4
Quinn	1024	3
Striegum	47	4
Svanhals	187	4
Trebi	936	4
Tonot	1012	2
Turkestan	711	1
Velvet	4252	4
Wider	1012	2
Zun Pohn Mugi		3
Unnamed	96	0
Unnamed	2416	2

TABLE 3. *Reaction to physiologic form 4 of F_3 plants from crosses between resistant and susceptible varieties*

Crosses	Hybrid no.	Number F_3 plants	
		Susceptible	Resistant
Chevalier C. I. 156 x Goldfoil C. I. 928	2-1	65	23
Trebi C. I. 936 x Goldfoil C. I. 928	4-1	8	4
" " " "	5-1	17	10
" " " "	5-2	14	5
" " " "	5-3	5	2
" " " "	5-4	3	2
" " " "	5-6	12	2
" " " "	5-7	19	3
Odessa C. I. 927 x Goldfoil C. I. 928	8-1	33	13
" " " "	8-2	69	20
" " " "	8-3	23	10
" " " "	8-4	29	8
" " " "	8-5	46	14
" " " "	8-6	39	8
" " " "	8-7	25	10
" " " "	8-8	15	9
" " " "	8-9	38	8
" " " "	8-10	37	9
" " " "	8-12	25	7
" " " "	8-13	9	3
" " " "	8-14	33	14
Goldfoil C. I. 928 x Velvet C. I. 4252	9-1	24	18
Observed totals		588	202
Calculated totals		592.5	197.5
Deviations		4.5	4.5

MILDEW REACTION OF THE F_3 PLANTS

Although the observed F_2 was close to the expectation and was entirely free from plants giving an intermediate reaction to powdery mildew (Plate I), the F_3 progeny tests confirmed the reaction of the F_2.

It was impossible to continue all F_2 plants through the F_3 progeny tests, because the individual F_2 plants were transplanted from the three-inch pots to the field in June and many did not mature. All the matured F_2 plants (126 in number) were harvested and used in these tests. These plants produced a total of 1,263 F_3 plants.

Forty-nine susceptible F_2 plants produced 414 susceptible F_3 and no resistant plants. These results are shown in table 4. Fifty-two susceptible F_2 plants produced 436 susceptible F_3 and 152 resistant F_3 plants. On the basis of a 3:1 ratio, the calculated number is 441:147 or a deviation of five plants from the actual results. Twenty-five resistant F_2 plants were apparently homozygous for resistance as they produced 261 resistant F_3 plants.

The largest number of F_3 individuals was the 1044 F_3 from 94 F_2 plants of the cross Odessa C.I. 927 x Goldfoil C.I. 928 (table 4). Forty-two susceptible F_2 plants were homozygous-susceptible, giving 372 susceptible F_3 plants. Forty-two susceptible F_2 segregated into 365 susceptible, to 122 resistant plants; 20 resistant F_2 plants weer homozygous-resistant as shown by their 185 resistant F_3 progeny.

TABLE 4. *Breeding behavior for mildew reaction of F_3 progenies of barley grown from seed of individual F_2 plants of crosses between susceptible and resistant varieties*

Cross	Hybrid no.	No. homozygous-susceptible		No. heterozygous			No. homozygous-resistant	
		Progenies	Plants	Progenies	Plants		Progenies	Plants
					Sus.	Res.		
Chevalier C.I. 156 x Goldfoil C.I. 928	2-1						2	51
Trebi C.I. 936 x Goldfoil C.I. 928	4-1	1	4	1	9	3	1	9
" " " "	5-1	3	12	4	30	10	1	3
" " " "	5-2			3	12	10	1	13
" " " "	5-3	1	8					
" " " "	5-6	1	2					
" " " "	5-7	1	16	2	20	7		
Odessa C.I. 927 x Goldfoil C.I. 928	8-1	4	35	5	29	11	2	10
" " " "	8-2	7	135	4	48	15	2	19
" " " "	8-3	2	14	6	39	10	1	9
" " " "	8-4	4	39	3	17	8	2	25
" " " "	8-5	2	9	9	105	37	2	32
" " " "	8-6	1	3	3	26	9		
" " " "	8-7	1	5					
" " " "	8-8	3	20	6	45	19	4	26
" " " "	8-9	7	38	3	29	5	4	23
" " " "	8-10	8	46				1	11
" " " "	8-12	1	10	1	12	4		
" " " "	8-13	1	13					
" " " "	8-14	1	5	2	15	4	2	30
Totals		49	414	52	436	152	25	261

SUMMARY

The response of 90 pure line varieties of barley to *Erysiphe graminis hordei* p.f. 4 was determined. The following four varieties were highly resistant: Goldfoil C.I. 928, Unnamed C.I. 96, Hanna C.I. 906 and Duplex C.I. 2433. Forty-four varieties were very susceptible and the other 42 were intermediate in reaction.

Susceptibility seems to be due to a single pair of factors as shown by the close approximation to a 3:1 ratio in the F_2 and verified by the F_3 progeny tests.

PLATE I

An F_2 plant resistant (left) to *Erysiphe graminis hordei* p. f. 4 and susceptible (right) of a cross Goldfoil C. I. 928 x Velvet C. I. 4252. This definite segregation is typical of the hybrid reaction to this physiologic form of powdery mildew.

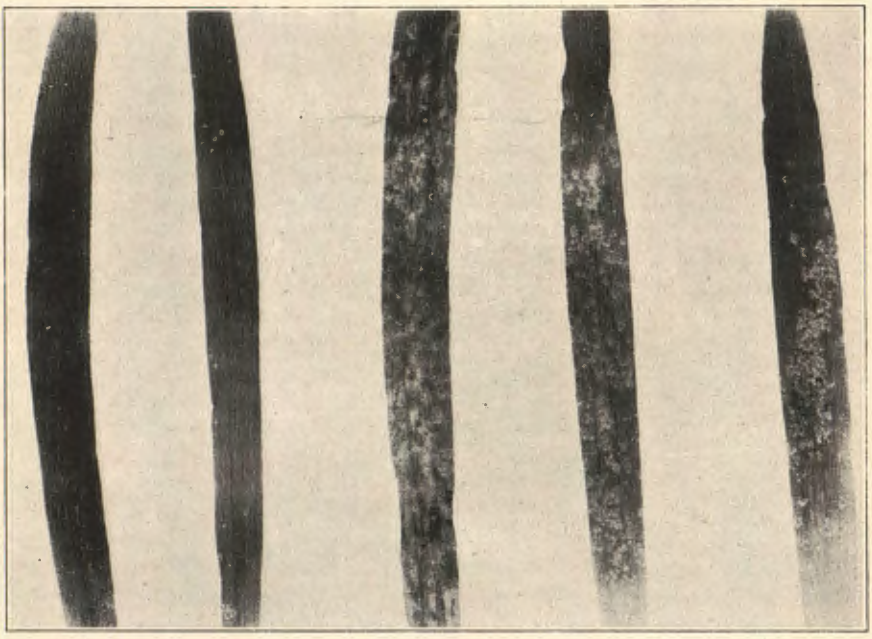

PLATE I

EXPERIMENTS ON THE BIOLOGY OF INFUSORIA INHABITING THE RUMEN OF GOATS[1]

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INTRODUCTION

In 1843, Gruby and Delafond, while performing experiments on digestion, discovered the protozoan fauna of the rumen and reticulum of domesticated ruminants. The main interest in the rumen infusoria centered at first in their morphology and classification, with occasional opinions expressed as to the physiological rôle in the host animals. Later investigations have dealt mainly with the latter phase of the subject. In a recent paper by Becker, Schulz and Emmerson (1930), the various views regarding the value of the rumen infusoria to their hosts have been discussed and the literature cited. They mention the following six outstanding opinions as to the significance of the presence of infusoria in the rumen: (1) The rumen infusoria convert plant substances within their bodies into animal substances which are more readily available to the digestion of the host; (2) they are useful as scavengers in reducing the numbers of bacteria and moulds that multiply in the rumen of the ruminants (bacteriophagic activities); (3) they are injurious parasites, causing pathological conditions in the digestive canal; (4) they aid mechanically in mixing the rumen contents; (5) they digest considerable amounts of cellulose, thus making it available to the host animals; (6) they are present merely as harmless commensals. In the course of their experiments, Becker, Schulz and Emmerson arrived at the conclusion that, until further investigations have definitely proved otherwise, the rumen infusoria must be regarded as mere harmless commensals. The research reported in this paper represents another method of approach to the problem of the significance of the host-parasite relationship in the instance under consideration.

REVIEW OF LITERATURE

Ferber (1928 and 1929) did some important work in determining the numbers of infusoria normally inhabiting the rumen under normal feeding conditions. For goats in a healthy condition and with normal feeding the number approximated 800 to 1,000 infusoria per cubic millimeter of rumen contents, or practically 1,000,000 per cubic centimeter.

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The animals and many of the materials used in these experiments were purchased from a grant made from the Bache Fund of the National Academy of Sciences.

[2]Indebtedness is acknowledged to Mr. John Zwiefel for his services in assisting with the care and handling of the goats used for the experiments.

For counting the infusoria he used a Fuchs-Rosenthal blood-corpuscle counting chamber. He found that the infusoria were extraordinarily sensitive to unfavorable conditions of nourishment. They disappeared within three or four days when the host was starved, but reappeared after four or five days with resumption of feeding. The infusoria seemed to disappear always in a certain order and to reappear in the reverse order of the disappearance. The infusoria appeared in lambs and kids only after they commenced to eat hay. In feeding adult animals with hay and water alone, however, the number of infusoria declined, only to rise again with the addition of more nourishing foodstuffs. Starch grains were apparently digested in the endoplasm through the agency of enzymes secreted by the rumen infusoria themselves, but the fat droplets of milk were digested inside the bodies of the infusoria through the action of the bacteria which had been ingested from the surrounding medium.

It has been repeatedly observed that the infusoria inhabit only the first two pouches of the ruminant stomach, and that those which pass into the third and fourth sections of the stomach are digested. With this fact in mind, Ferber (1928) experimented with the possibility of the infusoria utilizing non-albuminous foodstuffs and converting them into the protein of their own bodies, and thus increasing by this synthetic process the protein supply of their hosts. He fed the ruminants food rich in carbohydrates, but poor in protein, supplemented by the amides ammonium acetate and urea. He found that the infusoria could not use nitrogen-containing substances of a non-protein nature in the synthesis of their own protoplasm, for there was no subsequent increase in numbers such as followed the use of proteins as supplementary foods. Therefore, he denied the hypothesis that the infusoria synthesize proteins from amides.

He did believe, however, that the host derived benefit from the process of converting plant protein, difficult to digest, into animal protein, supposedly more easily digested. Certain physiological conditions of the hosts evidently call for increased protein metabolism. To help meet these requirements, there is an increase in the numbers of infusoria in the rumen.

How the physiological changes in the host condition the reproduction of the infusoria Ferber did not explain, but he assigned to the infusoria a symbiotic relationship with their ruminant hosts. This view was upheld by Mangold (1929,b), an eminent physiologist, who stated that the rumen infusoria played an important rôle in the nourishment of their host animals in that they transformed the plant protein, which is difficult to digest, into animal (infusorian) protein, which is more easily digested. In return for this service, the infusoria receive protection, shelter and food within the body of the host. This reciprocity represents a typical symbiosis.

At Mangold's instigation, Ferber (1929, a) carried on further experiments as to the effects on the infusoria of variations in the protein metabolism of the host animals. He found that, during the last six weeks of pregnancy and during the lactation period, the number of infusoria in goats and sheep increased to double the usual number—that is, to around 2,000 per cmm. of rumen contents, and that, after lactation ceased the infusoria returned to the normal number of about 1,000 per cmm. This heavy increase in the infusoria numbers concomitant with the increased

protein metabolism of the host, he affirms, represents the contribution of the infusoria to the support of this increased protein metabolism.

Ferber included in his investigation observations on young, growing lambs and kids in which there is, of course, a more active protein metabolism than when the mature condition is attained. The infusoria appear in the rumens of the lambs and kids coincident with the consumption of hay as food, before the cessation of suckling. During the period of rapid growth of the kids, especially from the fifth to the seventh or eighth month, the inusoria increase to double the normal number, and then, with the diminishing growth rate of the host, decrease to normal with the attainment of the adult size. The situation is thus analagous to that in pregnant and lactating animals. Experiments were performed also upon lambs of the same age, but in different conditions of nourishment. For these observations Ferber employed lambs on pasture. At the time of obtaining the rumen samples, he noted whether the lambs, in the judgment of the shepherd who cared for and knew them, were in a condition of good, medium or poor nourishment. Here again it was found that high numbers of infusoria coincide with good and medium states of nutrition, and that poorly nourished animals have the lowest population of rumen infusoria. Still further, he found that the nourishment condition and infusoria numbers were still in accord in animals of varying ages, with, in general, a decrease in infusorian numbers accompanying an increase in age. In normally nourished, slow-growing, or adult animals, the medium, normal number of infusoria was fairly constant, around 1,000 per cmm. of rumen contents.

Ferber deduced from these results that there must be an optimal condition of the rumen contents for the well-being of the animal, and that this condition was reflected in the number of infusoria present therein. He, accordingly, investigated the relationship between the rumen infusoria and the density of the rumen content (1929, b). The conditions of density of the rumen contents were designated as "normal", "thick", "thin", "very thick", and "very thin". The results yielded some irregularities, but in general with a "normal" density, the number of infusoria were "normal"; with "thick" contents, there was an increase in infusoria; with "thin", there was a decrease. The hydrogen ion factor also entered here, the pH with normal and increased numbers of infusoria being around 7.9; while a drop to the acid side, even to 6.9, accompanied a great loss in the infusorian population. It was inferred, therefore, that there is an optimal condition of the rumen contents in which the infusoria, as well as the host animals, find the most favorable conditions. He further stated that, since in any instance of symbiosis the optimal success of one member is dependent upon that of the other, the number of infusoria could be considered as an indicator of the well-being of the host. He felt that the consistency of the rumen contents and the protozoan population therin were of interest for the practical nutrition of animals, for with favorable cooperation and regulation of these factors, their highest possible productive capacities could be reached.

Inspired by the experiments of Schwarz (1925), who also held to the theory of a symbiotic relationship between the infusoria and their hosts, Ferber and Winogradowa-Fedorowa (1929) tried to achieve a quantitative

determination of the amount of the rumen infusoria. When Gruby and Delafond (1843) discovered the presence of the infusoria in the rumen of ruminants, they estimated that the weight of the protozoa equalled approximately one-fifth of the total weight of the rumen content. Ferber and Winogradowa-Fedorowa found that the total mass of infusoria amounted to a twelfth to a twentieth part of the entire rumen content, and that the entire infusorian nitrogen amounted to from 10 to 20 per cent of the nitrogen present in the rumen. From these results Ferber computed the infusorian protein mass to be 155.6 g. per 100 kg. of rumen contents, in contrast to the higher figure of 256 g. as given by Schwarz. Since the number of infusoria remained fairly constant, and they found an average of seven per cent division forms each day, they drew the conclusion that seven per cent of the total number of infusoria must disappear daily to be digested in the psalterium. They computed therefrom that the rumen infusoria yielded daily to their host animals (sheep and goats) an average of 0.327 g. of protein and that the infusorian protein digested by the host amounted to about two per cent of the total protein digested daily. After three days of starvation of the host, the infusoria disappeared, and, in reappearing with resumed feeding, divided most rapidly on the first day, with the division rate then gradually declining again to the normal percentage of seven per cent.

In presenting the subject of nutrition and digestion in ruminants, Mangold (1929, a) reiterates the importance of the rumen infusoria for their host animals. He agrees that the infusoria reduce cellulose, and show an active carbohydrate assimilation, digesting both starch (Trier, 1926) and fat (Ferber, 1928); that they facilitate protein utilization by transforming plant protein into the more easily digestible animal protein of their own body substance; that the infusoria need protein and are not to be satisfied with amides which they are not themselves able to manufacture into protein. In addition, Mangold (1929, b) states that the number of infusoria within the rumen of the host is limited in a physiological manner under ordinary conditions of good health in the host animal. He considers that the symbiotic relationship between the rumen infusoria and the ruminants has practically been proved. He feels that, since the parallel between the amount of protein metabolism of the host and the infusoria number is an incontestable fact, the increased protein requirement of the host primarily causes the increase in the number of protozoa. But he also states that the physiological connection between the two is as yet unexplained, and that there remains yet to be solved what physiological changes occur in the rumen which, as soon as the protein metabolism increases, cause the rise in the number of infusoria; and conversely, what physiological factors, in the case of decrease of the protein metabolism, cause the lowering of the infusoria numbers.

THE INVESTIGATION

PURPOSE OF THE RESEARCH

The investigation was undertaken in order to make additional contributions to facts already known concerning conditions which regulate the increase or decrease of infusorian population in the paunch of the ruminant stomach. Although practical applications of the results were not

foremost in mind at the time of selecting the problem, these would, of course, be evident in case it should be shown that the infusoria in question are material aids to their hosts. Finally, the investigation was planned with the view that a critical analysis and interpretation of the results might yield information either confirmatory or otherwise to the views of Ferber and of Mangold that the relationship between host and parasite is one of symbiosis or mutual aid. A reinvestigation of the problem from the standpoint of the biology of the infusoria was necessary, especially in view of the results in general contradictory to these claims obtained by Becker, Schulz, and Emmerson (1930) and by Becker and Everett (1930).

METHOD OF PROCEDURE

Statement of the Problem

The general problem is stated in the preceding paragraph. More explicitly, the various aspects of the problem were the determination of the following points: (1) the effects due to kind and amounts of a variety of food materials upon the numbers of infusoria; (2) the effects of the food upon the pH of rumen contents and the consequent effect upon the infusoria; and (3) the relationship between the amount of sediment and the number of infusoria.

For these experiments three goats were used, one female of about four to five years of age, and two males, each of about one to two years. In the tables, the female goat is designated as Goat I, and the two males as Goat II and Goat III, the latter being the larger of the two animals. The female goat freshened during the course of the investigation, giving birth to two kids. Thus we were enabled to obtain data during the periods of pregnancy and lactation of this one goat.

Species of protozoa present. The infusoria present in the three goats varied somewhat as follows: In Goat I, *Diplodinium multivesiculatum, D. ecaudatum, D. hamatum, Entodinium simplex, E. minimum, E. caudatum, E. furca, and E. bicarinatum;* in Goat II, *D. multivesiculatum, D. bursa, D. ecaudatum, E. caudatum, E. bicarinatum, E. simplex,* and *E. minimum;* while in Goat III there were still fewer species, namely, *D. multivesiculatum, D. ecaudatum,* and *E. minimum,* with a few *E. simplex,* and very rarely an *E. caudatum.* About the middle of October, 1929, the species of Diplodinium above mentioned disappeared in all three goats, except *D. multivesiculatum,* which was, from then on, the only species of Diplodinium represented. The Entodinium fauna remained about the same for each goat throughout the period of the experiments. The genera Ophryoscolex, Isotricha, Dasytricha and Buetschlia were not represented in the infusorian fauna of the three goats used in these investigations.

Experimental Methods

Obtaining samples. In obtaining samples of rumen contents for examination, the jaws of the goat were held apart by means of a wooden block inserted across the mouth between the teeth. A rubber tube (horse catheter) was passed through a hole in the center of the block and pushed down the oesophagus into the rumen. Care had to be exercised that the tube did not pass into the trachea. When in the rumen, suction was ap-

plied to the tube, and the sample of rumen contents thus obtained was expelled into a bottle.

Feeding. The hay and grain were eaten readily by the goats. It was necessary, however, to prepare and inject into the goats the other materials used, in order to assure the intake of a definite quantity of them. A suspension was made by mixing and stirring the material with tap water. This was passed into the rumen through a funnel connected to a rubber tube. The asparagin was ground in a mortar, but did not go into solution when water was added. The fine particles were washed through the tube into the rumen with water.

Determining pH. Immediately upon reaching the laboratory, the sample of rumen contents was thoroughly shaken and a definite quantity transferred to a vial by means of a marked pipette. This sample was then diluted with twice the volume of distilled water. The two liquids were thoroughly mixed, and the hydrogen ion concentration of the diluted rumen content was determined at first by both the La Motte Roulette comparator and the drop method. The results obtained compared so closely that after a few weeks only the drop method, which was quicker and easier, was used. Phenol red was the indicator employed throughout.

Counting and computing. For counting the infusoria, a Max Levy haemocytometer and an ocular micrometer calibrated to the microscope used were employed. From the thoroughly mixed mass of rumen content and distilled water a drop was transferred, by means of a pipette, to the counting chamber, and the infusoria which fell within the square marked on the ocular micrometer were counted. Account was taken only of the numbers of the two genera, Diplodinium and Entodinium, present. The counting chamber was shifted so that counts were made from six different locations in each drop. The chamber was refilled four times, giving a total of twenty-four counts for each sample of rumen content. The total numbers of Diplodinium and of Entodinium thus found were multiplied by three to account for the dilution of the sample, and then by six and two-thirds, which was necessary, according to the calibration previously determined, to express the total amount in cubic millimeters. Since twenty-four counts were taken, the average number per cubic millimeter was obtained by dividing the total amount by twenty-four, and the average number per cubic centimeter by multiplying by 1,000.

Determining percentage of sediment. The remainder of the sample of rumen content which was not diluted and used in the counting was poured into a test-tube and left to stand, so that a sediment would form. The percentage by volume of sediment to the total amount was then calculated.

Determining volume of protozoa. Measurements of fifty Entodinia were made. The specimens were taken as they were encountered with no regard for species or for size. Fifty *Diplodinium multivesiculatum* were measured. An ocular micrometer which had been calibrated to the microscope was employed. The specimens were measured for length, width and thickness. Length and breadth were easy to measure, but to obtain the thickness it was necessary to tap the covre glass gently until the specimen being measured was turned. After securing the fifty sets of measurements for each genus, the averages were computed for the three dimensions for both genera. Models of plastic clay were then constructed according to

scale for the average measurements of the two genera, and the volumes of each determined by the displacement of water.

PRESENTATION OF DATA

Different Feeds and Tables of Results

Effects on numbers of protozoa of feeding fresh green plants. At first the goats were allowed to graze on blue grass within the confines of a pen. While on this feed, they drank little or no water. Table 1-a shows the numbers of infusoria after several days of this diet. The experiments were begun on Goat I in August, 1929, while Goats II and III were not used until September, 1929. In most cases the diet given to one goat at one time was repeated upon another at a later date, if not during the same interval. Table 1-b shows the results of feeding green alfalfa. With these feedings on green fodder, the infusoria number remained surprisingly low, averaging between 250,000 and 300,000 per cc.

Effects with green fodder and grain. Goats II and III were given 500 grams of grain mixture in addition to the blue grass on which they grazed at will. The grain mixture used consisted of 100 parts of ground corn, 100 parts of ground oats, 50 parts of wheat bran, 10 parts of linseed

TABLE 1 *Effects of feeding fresh green plants*

Day	Goat	pH	No. of Ento-dinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
(a) Blue grass *ad libitum*									
9th	I	8.0	232,915		50,416		283,331		
10th	I	7.8	177,500	0.381	18,333	6.312	195,833	239,582	6.693
5th	II	7.5	189,167		7,083		196,250		
6th	II	7.6	162,500		10,000		172,500		
8th	II	7.8	265,417		23,333		288,750		
9th	II	7.6	267,917	0.411	35,000	3.462	302,917	240,104	3.873
5th	III	7.6	270,000		4,167		274,167		
6th	III	7.4	296,667		5,000		301,667		
8th	III	7.4	296,250		7,500		303,750		
9th	III	7.1	98,750	0.446	.....	0.765	98,750	244,583	1.211
(b) Green alfalfa *ad libitum*									
4th	I	7.8	360,833		55,000		415,833		
5th	I	8.0	411,666		60,000		471,666		
7th	I	8.2	360,833		75,000		435,833		
8th	I	8.0	722,916	0.816	110,000	13.772	832,916	539,062	14.633
7th	II	7.6	130,000		14,166		144,166		
8th	II	7.7	259,166		17,500		276,666		
9th	II	7.6	215,833		25,000		240,833		
10th	II	7.6	204,166		30,000		234,166		
11th	II	7.7	207,500	0.377	23,333	4.040	230,833	225,333	4.417

TABLE 2 *Effects of feeding with green fodder and grain*

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
(a) Blue grass *ad libitum* and 500 g. grain									
5th	II	7.5	380,833		65,833		446,666		
6th	II	7.4	284,166		89,166		373,332		
8th	II	7.5	147,916		129,166		277,082		
10th	II	7.3	132,500	0.439	11,666	13.581	144,166	310,289	14.020
5th	III	7.2	1,645,833		30,833		1,676,666		
6th	III	7.2	1,215,000		14,166		1,229,166		
8th	III	7.1	776,666		55,833		832,499		
10th	III	7.1	925,833	2.119	15,833	5.356	941,666	1,169,999	7.475
(b) Green alfalfa *ad libitum* and 1000 g. grain									
22nd	I	7.6	2,999,167		89,167		3,088,334		
23rd	I	7.8	2,447,500		90,833		2,538,333		
25th	I	7.7	3,014,583		115,833		3,130,406		
26th	I	7.5	3,900,833	5.736	173,333	21.538	4.074,166	3,207,812	27.274

oil meal, and some minerals. Table 2-a shows the effects of this combination upon the numbers of protozoa, and table 2-b those of the green alfalfa-grain combination. As these two tables clearly indicate, the addition of the grain mixture to the green feed greatly increased the infusorian population.

Effects with grain. Table 3 showing the effects of feeding grain alone, depicts a lowering of the pH, and, after an initial increase, a considerable decrease of the infusorian numbers. The decrease may have been due to the pH, but it should be stated that the goat tired of the diet of grain alone, and, after the first week, ate only about one-half of the 1,000 grams offered her daily.

TABLE 3 *Effects of feeding with grain alone*

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
1,000 g. grain									
5th	I	6.9	6,707,500		75,000		6,782,500		
6th	I	7.0	7,520,833		76,666		7,597,499		
8th	I	7.0	150,833		9,166		159,999		
10th	I	7.0	497,750	6.903	12,166	7.941	509,916	3,762,478	14.844

The goats were fed for the remainder of the experiments on dried alfalfa hay, used alone, or in conjunction with other foodstuffs. Tap water, unless otherwise stated, was given for drink. The daily count showed that an interval of seven to nine days after the instalment of a new feed was necessary for the infusorian fauna to reach a more or less constant level.

Effects with alfalfa hay. As indicated in table 4, the infusorian fauna remained at a lower level with a feed of dried hay than with green fodder, but rose with the addition of grain, increasing with increased amounts of grain.

TABLE 4 *Effects of feeding with alfalfa hay*

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
(a) 250 g. hay									
5th	II	7.5	94,166		2,500		96,666		
6th	II	7.9	79,166		4,166		83,332		
7th	II	7.8	115,816		10,816		126,632		
8th	II	7.7	101,666	0.181	4,166	0.534	105,832	103,115	0.175
(b) 500 g. hay								....	
6th	I	7.6	75,000		15,833		90,833		
7th	I	7.6	112,500		12,500		125,000		
8th	I	7.6	91,666		16,666		108,332		
9th	I	7.6	75,000	0.164	10,000	2.525	85,000	102,791	2.689
7th	II	7.8	206,666		13,333		219,999		
8th	II	7.7	275,833		10,000		285,833		
10th	II	7.7	247,500		10,833		258,333		
11th	II	7.8	270,000		8,333		278,333		
12th	II	7.7	280,833	0.475	5,833	1.775	286,666	265,833	2.250
6th	III	7.2	102,500		1,666		104,166		
7th	III	7.5	99,166		5,000		104,166		
8th	III	7.5	105,000		5,816		110,816		
9th	III	7.5	102,500	1.190	10,833	1.070	113,333	108,120	1.260
(c) 1,000 g. hay									
8th	I	7.8	142,500		21,666		164,166		
10th	I	7.7	95,833		11,666		107,499		
11th	I	7.8	84,166		3,333		87,499		
12th	I	7.7	109,166	0.200	4,166	1.874	113,332	118,124	2.074
8th	III	7.6	137,500		8,333		145,833		
10th	III	7.7	140,833		10,000		150,833		
11th	III	7.6	135,000		5,000		140,000		
12th	III	7.7	192,500	0.281	24,166	2.180	216,666	163,333	2.461

TABLE 5 *Effects of feeding with alfalfa hay and grain*

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
(a) 400 g. hay and 250 g. grain									
7th	I	7.8	1,903,333		27,500		1,930,833		
8th	I	7.8	1,566,666		40,833		1,607,499		
9th	I	7.8	1,975,833		63,333		2,039,166		
10th	I	7.7	2,043,333	3.474	20,833	7.000	8,064,166	1,910,416	10.474
(b) 500 g. hay and 125 g. grain									
8th	II	7.7	983,333		20,000		1,003,333		
9th	II	7.7	1,265,833		39,166		1,304,999		
10th	II	7.7	1,368,333		25,000		1,393,333		
11th	II	7.7	1,612,500		29,375		1,641,875		
13th	II	7.7	1,622,500	2.524	35,000	5.455	1,657,500	1,400,208	7.979
(c) 500 g. hay and 250 g. grain									
4th	II	7.5	7,795,000		67,500		1,862,500		
5th	II	7.6	1,705,833		62,500		1,768,333		
6th	II	7.6	1,605,000		25,833		1,630,833		
7th	II	7.6	2,384,166	3.475	85,833	11.094	2,469,999	1,932,916	14.569
(d) 1,000 g. hay and 125 g. grain									
8th	I	7.7	687,500		2,500		690,000		
9th	I	7.7	967,500		1,666		969,166		
10th	I	7.7	991,666		3,333		994,999		
11th	I	7.7	890,000		.....		890,000		
13th	I	7.7	597,500	1.534	1,666	0.336	599,166	828,666	1.870
8th	III	7.5	430,833		21,666		452,499		
9th	III	7.5	382,500		17,500		400,000		
10th	III	7.5	382,500		10,000		392,500		
11th	III	7.5	451,666		43,333		494,999		
13th	III	7.5	315,833	0.729	10,416	3.780	326,249	413,249	4.509
(e) 1,000 g. hay and 250 g. grain									
5th	I	7.7	1,423,333		1,666		1,424,999		
6th	I	7.7	1,121,666		2,500		1,124,166		
8th	I	7.7	2,296,666		4,166		2,300,832		
9th	I	7.7	2,230,833	3.281	2,500	0.479	2,233,333	1,770,832	2.778
5th	III	7.5	383,333		71,666		454,999		
6th	III	7.5	352,500		90,833		443,333		
8th	III	7.5	285,000		11,666		296,666		
9th	III	7.5	349,166		37,500		386,666		
10th	III	7.5	468,333	0.682	53,333	9.742	521,666	420,666	10.424
(f) 150 g. hay and 375 g. grain									
7th	III	7.0	810,000		59,166		869,166		
8th	III	7.0	970,000		100,000		1,070,000		
10th	III	7.0	1,540,000		39,166		1,579,116		
11th	III	7.0	2,745,000	2.814	52,500	11.492	2,797,500	1,578,958	14.306

TABLE 5 (Cont.)

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
(g) 250 g. hay and 375 g. grain									
7th	I	7.2	3,124,500		90,833		3,218,333		
8th	I	7.2	3,010,000		105,833		3,115,833		
10th	I	7.2	2,487,500		65,833		2,553,333		
11th	I	7.2	2,881,666	5.338	118,333	17.483	2,999,999	2,971,874	22.821
7th	II	7.0	4,408,333		26,666		4,434,999		
8th	II	7.0	4,465,000		66,666		4,531,666		
10th	II	7.0	5,597,500		86,666		5,684,166		
11th	II	7.0	4,947,500	9.010	52,500	10.673	5,000,000	4,912,707	19.683
(h) 500 g. hay and 375 g. grain									
6th	II	7.6	4,757,500		127,500		4,885,000		
7th	II	7.6	3,314,166		184,166		3,498,322		
8th	II	7.6	2,389,166		123,333		2,512,499		
10th	II	7.6	1,655,000	5.622	70,000	23.183	1,725,000	3,155,205	28.805
6th	III	7.7	2,200,833		40,833		2,241,666		
7th	III	7.7	2,149,166		82,500		2,231,666		
8th	III	7.7	1,367,500		65,000		1,432,500		
10th	III	7.7	1,103,333	3.165	46,666	10.788	1,149,999	1,763,955	13.953
(i) 750 g. hay and 375 g. grain									
6th	I	7.7	1,970,333		14,166		1,984,499		
7th	I	7.8	2,150,000		29,166		2,179,166		
8th	I	7.8	2,288,333		39,166		2,327,499		
10th	I	7.8	1,601,666	3.717	44,166	5.815	1,645,832	2,034,249	9.532
(j) 750 g. hay and 60 g. grain									
8th	I	7.7	815,833		35,833		851,666		
9th	I	7.8	725,833		24,166		749,999		
10th	I	7.8	740,000		34,166		774,166		
11th	I	7.8	746,666	1.405	23,333	4.394	769,999	786,457	5.799
(k) 500 g. hay and 60 g. grain									
8th	II	7.7	1,680,833		60,000		1,740,833		
9th	II	7.8	1,818,333		62,500		1,880,833		
10th	II	7.8	1,540,000		70,833		1,610,833		
11th	II	7.8	1,155,000	2.874	40,000	10.712	1,195,000	1,606,875	13.586
8th	III	7.7	956,666		33,333		989,999		
9th	III	7.8	1,105,000		33,333		1,138,333		
10th	III	7.8	1,081,666		35,000		1,116,666		
11th	III	7.8	903,333	1.877	46,666	6.763	949,999	1,048,249	8.640

Effects with alfalfa hay and grain. The different combinations of hay and grain fed are indicated in the different sections of table 5. The drop in numbers shown for both goats in section d may have been occasioned by the small amount of grain in proportion to the hay given as feed. With an increased amount of grain and the same amount of hay,

the numbers rose again for Goat I, which ate all the food given her, but remained low for Goat III, whose appetite was not sufficient at the time to consume the full allotment of his daily feed (section e).

Judging from the results given in sections f, g, h, and i of table 5, 375 grams of grain supplied optimal conditions for the growth of the infusoria, as the numbers remained very high, even with varying amounts of hay. With an increased amount of hay (section h), the numbers remained high. Goat III was especially irregular in his feeding, eating all the hay on some days and very little on others. All of the goats were always

TABLE 6 *Effects of feeding with hay, grain, tap and distilled water*

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
(a) Hay ad *libitum*, 250 g. grain, and tap water									
6th	I	7.7	954,166		85,833		1,039,999		
7th	I	7.9	825,833		51,666		877,499		
8th	I	7.7	1,085,833	1.733	38,333	10.763	1,124,166	1,013,888	12.536
6th	II	7.8	775,000		47,500		822,500		
7th	II	7.8	595,000		50,833		645,833		
8th	II	7.7	1,046,666	1.495	63,333	9.895	1,109,999	859,444	11.390
6th	III	7.0	710,833		4,166		714,999		
7th	III	7.2	598,833		5,833		604,666		
8th	III	7.7	884,166	1.356	23,333	2.040	907,499	742,388	3.396
(b) Hay *ad libitum*, 250 g. grain, and distilled water from glass jar									
4th	I	7.8	1,057,500		65,833		1,123,333		
6th	I	7.7	797,500		52,500		850,000		
8th	I	7.7	984,166		35,000		1,019,166		
10th	I	7.7	1,270,833	1.907	37,500	8.760	1,308,333	1,075,208	10.667
4th	II	7.7	1,479,166		59,166		1,538,332		
6th	II	7.6	2,448,333		87,500		2,535,833		
8th	II	7.7	2,124,166		102,500		2,226,666		
10th	II	7.6	2,154,166	3.817	97,500	15.868	2,251,666	2,138,124	19.685
4th	III	7.7	839,166		70,833		909,999		
6th	III	7.4	783,333		47,500		830,833		
8th	III	7.6	782,500		49,166		831,666		
10th	III	7.7	600,833	1.394	35,833	9.334	636,666	802,291	10.728
(c) Hay *ad libitum*, 250 g. grain, and distilled water from tin bucket									
4th	I	7.6	1,275,833		72,500		1,348,333		
6th	I	7.6	931,666	1.863	43,333	10.635	974,999	1,161,666	12.498
4th	II	7.6	2,595,833		90,833		2,686,666		
6th	II	7.6	2,526,666	4.754	85,833	16.220	2,612,499	2,649,582	20.974
4th	III	7.5	1,056,666		34,166		1,090,832		
6th	III	7.5	848,333	2.716	42,500	7.039	890,833	990,832	9.755

TABLE 7. *Effects of two days of starvation*

No food. Tap water only.

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sediment
3rd	II	7.3	640,000	1.187	15,833	2.907	655,833	655,833	4.094	40.97
3rd	III	7.6	62,500	0.116	.....	.....	62,500	62,500	0.116	11.58

TABLE 8. *Effects of reinstated feeding with hay and grain*

(a) 227 g. hay and 150 g. grain

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sediment
4th	II	7.7	494,166		29,166		523,332			67.35
5th	II	7.7	972,500		59,166		1,031,666			63.64
7th	II	7.7	1,200,000		88,333		1,288,333			50.00
7th	II	7.7	1,408,333		94,166		1,502,499			53.57
8th	II	7.7	1,645,833		76,666		1,722,499			46.15
11th	II	7.6	320,000		79,166		399,166			71.43
12th	II	7.6	372,500		66,666		439,166			94.29
13th	II	7.6	558,333		39,166		597,499			90.91
14th	II	7.5	805,000		68,750		873,750			72.09
15th	II	7.7	1,078,333		51,666		1,129,999			55.56
18th	II	7.7	1,455,833	1.739	61,666	11.929	1,517,499	1,002,309	13.668	45.83
4th	III	7.2	206,666		2,500		209,166			80.65
5th	III	7.4	331,666		17,500		349,166			91.18
6th	III	7.7	299,166		23,333		322,499			.....
7th	III	7.8	380,833		26,666		407,499			51.11
8th	III	7.4	215,833		17,500		233,333			58.33
11th	III	7.7	678,333		39,166		717,499			58.06
12th	III	7.7	830,833		60,000		890,833			51.52
13th	III	7.6	830,833		58,333		663,333			55.19
14th	III	7.5	860,833		50,000		910,833			76.19
15th	III	7.8	438,333		33,333		471,666			43.14
18th	III	7.6	210,833	0.853	14,166	5.717	224,999	490,984	6.570	35.26

(b) 454 g. hay and 300 g. grain

5th	II	7.7	2,805,000		80,000		2,165,000			66.67
8th	II	7.7	2,061,666		60,000		2,121,666			68.00
10th	II	7.7	2,525,000	4.127	70,000	12.854	2,595,000	2,293,888	16.981	48.78
5th	III	7.6	1,500,000		55,000		1,555,000			27.70
8th	III	7.6	1,438,333		61,666		1,499,999			44.64
10th	III	7.6	1,215,000	2.569	41,666	9.691	1,256,666	1,437,222	12.260	37.50

very anxious to receive their grain allotment. With a reduction in the amount of grain given, the protozoan fauna decreased again in number (table 5, sections j and k).

Effects with alfalfa hay, grain and distilled water. While performing some previous experiments on rumen infusoria in goats and sheep, Dr. Becker had noticed a decided drop in numbers with the use of distilled water for drink. He mentioned this fact to the writer, and experiments were performed to determine what effect the distilled water would have. Table 6-a shows the numbers of infusorian fauna in the goats before the use of the distilled water for drinking purposes. Section b indicates that the distilled water had no unfavorable effect upon the numbers of protozoa. In his earlier noting of the drop of infusoria numbers with the drinking of distilled water, a tin bucket had been used as the container. In the experiments tabulated in table 6-b the distilled water was administered from a glass jar. As it was thought that the tin bucket might have had some toxic effect previously, one was again used in place of the glass jar. In this case, however, there was apparently no toxic effect from the tin, as the numbers increased, as is indicated in table 6-c. Consequently the deleterious effects attributed to the distilled water must have been due to some other factor.

Effects of starvation. Goats II and III were starved for two days after the experiments with the distilled water, and were given nothing but tap water to drink. Table 7 shows the great decrease in the infusoria numbers after the starvation period, and indicates the rapidity with which the protozoan fauna falls off under adverse conditions.

Effects of reinstated feeding with hay and grain. With reinstated feeding of hay and grain, the numbers rose again (table 8-a), with some unexplainable drops, however. With a doubling of the food (table 8-b), the numbers increased to approximately the same level as in table 5-h, when slightly more food was given. In both cases, the goats had good appetites, and ate all the food given them.

Effects with hay. According to Ferber (1928), hay and water alone as food caused a decrease in the numbers of protozoa. Table 9 shows the agreement of our results with those obtained by him.

Effects with hay supplemented by cracked corn. The hay diet was supplemented by carbohydrate food in the form of cornstarch, and this combination was used either alone, or as a basis for additional foodstuffs, until the end of the experiments. There was one exception to this, however, in April, 1930, when Goat III was put on a diet of hay alone, and then of hay supplemented by cracked corn. The infusorian numbers in this case (table 10) practically coincide with those for the apparently optimal amounts of hay and grain. (Compare tables 5-h and 8-b).

Effects with hay supplemented by carbohydrate food. The cornstarch used to supplement the hay for the carbohydrate food was Argo Corn Starch, produced by the Corn Products Refining Company of Edgewater, New Jersey. The Research Department of the company very kindly submitted the following average analysis of the corn starch, figured on dry substance: starch, 98.29 per cent; protein, 0.33 per cent; ash, 0.13 per cent; fat, 0.55 per cent; soluble, not protein, 0.07 per cent. From 0.2 per cent to 0.3 per cent pentosans may also be present.

In giving the cornstarch as food, one-half pound of the starch was mixed with about a quart of tap water, and the suspension thus formed

TABLE 9. *Effects of omitting the grain and feeding with hay alone*

681 g. hay

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sediment
13th	II	7.2	280,833		10,000		290,833			32.14
14th	II	7.7	329,166		13,333		342,499			23.53
15th	II	7.7	315,000		17,500		332,500			36.74
17th	II	7.8	249,166	0.545	7,500	2.219	256,666	307,374	2.764	9.21
13th	III	7.2	356,666		28,333		384,999			82.42
14th	III	7.4	250,000		18,333		268,333			38.48
15th	III	7.6	185,000		10,833		195,833			48.57
17th	III	7.7	218,333	0.468	9,166	3.060	227,499	269,166	3.528	23.91

TABLE 10. *Effects of feeding with cracked corn supplementing the hay*

681 g. hay and 340 g. cracked corn

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sediment
8th	III	7.1	1,600,833		2,500		1,603,333			72.00
10th	III	6.7	1,133,333		6,666		1,139,999			45.21
11th	III	6.8	1,616,666		27,500		1,644,166			65.71
13th	III	7.2	862,500		110,000		972,500			78.05
15th	III	7.4	986,666	2.301	147,500	10.803	1,134,166	1,298,833	13.104	.....

TABLE 11. *Effects of feeding with hay supplemented by carbohydrate food*

681 g. hay and 227 g. cornstarch

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sediment
9th	II	7.7	388,333		38,333		426,666			34.78
10th	II	7.7	315,000		62,500		377,500			15.80
11th	II	7.7	245,000		36,666		281,666			21.62
16th	II	7.8	405,833		86,666		492,499			39.47
17th	II	7.7	652,500		101,666		754,166			62.50
18th	II	7.7	843,333		100,000		943,333			34.88
4th	II	7.8	680,833		22,500		703,833			18.60
5th	II	7.8	905,000		45,000		950,000			23.53
6th	II	7.6	1,135,833		21,666		1,157,499			19.61
8th	II	7.9	1,469,166	1.306	52,500	10.423	1,521,666	760,832	11.729	33.33
9th	III	7.7	536,666		.....		536,666			56.00
10th	III	7.8	585,000		1,666		586,666			69.23
11th	III	7.7	776,666		4,166		780,832			73.53
16th	III	7.6	849,166		23,333		872,499			64.15
17th	III	7.4	584,166		8,333		592,499			40.54
18th	III	7.1	665,000	1.226	34,166	2.193	699,166	678,055	3.419	49.02

poured through a funnel in the end of the stomach tube, and thus into the rumen of the goat. With the addition of the starch to the hay diet the numbers of protozoa increased markedly. (Compare table 2 with tables 9 and 4). Mangold (1929, b) declared that there was no increase in the protozoan fauna with the use of carbohydrate food; that food rich in carbohydrates could in no way compensate for a lack of plant protein which he stated stimulated the infusorian increase. The "starch level" attained in these experiments (see table 11) shows the numbers more than doubled those for the "hay level", although they fall far below those of the level for hay and grain.

Effects with hay and starch supplemented by protein foodstuffs. Following the above experiment, protein foodstuffs were added to the same diet of hay and cornstarch to determine the effects of the proteins upon the numbers of infusoria. The various foodstuffs were mixed with the cornstarch and poured into the animal in suspension as above described. A small quantity of Loeffler's dehydrated beef blood serum increased the protozoan fauna rapidly, in opposition to Mangold's conclusion that it is plant protein which stimulates the increase. With the addition of egg albumin, Merck, impalpable powder, the protozoan number remained at about the "starch level", for Goat II. (Tables 11 and 12-b).

Goat II alone was used for the remaining experiments because Goat III lost his appetite and was discarded. The wheat gluten flour supplementing the hay and cornstarch was very favorable for the development of the infusoria, as the numbers increased to nearly double the numbers on hay and starch alone. Table 12-c shows the increase during this diet, which, however, did not reach the level attained by the same goat with the dehydrated blood serum. Supplementing the feed with asparagin (table 12-d) did not promote the multiplication of the infusoria since the figures obtained fall well within the probable error for starch and hay alone. With casein (table 12-e) the numbers rose higher, though they did not reach the high level obtained with the addition of dehydrated beef blood serum to the hay and cornstarch. To summarize, table 12, with the results of a hay and cornstarch diet supplemented by protein foodstuffs, shows a significant increase in the numbers of protozoa, except in the cases of the egg albumin and of asparagin which is, of course, not a protein.

Effects of pregnancy. After the experiments with the distilled water (table 6), Goat I was kept on the same feed for a few days longer, with tap water to drink (table 13-a). Then her food was doubled in amount (table 13-b) to meet the demands of her pregnant condition. This caused an increase in the protozoan numbers, though the number was not consistently double the normal. On February 19, 1930, she gave birth to two kids, one of which survived.

Effects of lactation. Table 14 depicts the effects of the lactating period upon the numbers of infusoria in the rumen, and shows that, for this goat, the level during that period was even slightly higher than that indicated by Ferber (1929, a). About the end of April the kid began eating considerable amounts of the mother's grain. This factor had an influence on the protozoan numbers, as table 14-b indicates in the average given under the 112th day. The kid was taken from the mother goat on

TABLE 12. *Effects of feeding with hay and cornstarch supplemented by protein*

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sediment
(a) 681 g. hay, 227 g. cornstarch and 30 g. Loeffler's dehydrated beef blood serum										
7th	II	....	2,836,666		65,833		2,902,499			.....
8th	II	7.5	2,715,000		53,333		2,768,333			.....
10th	II	7.7	2,928,333		35,000		2,963,333			46.15
11th	II	7.8	2,705,000	5.190	45,833	9.181	2,750,833	2,846,249	14.371	33.33
9th	III	7.8	1,614,166		60,000		1,674,166			90.00
10th	III	7.8	1,282,500		50,000		1,332,500			79.16
12th	III	7.9	1,524,166		35,000		1,559,166			48.15
14th	III	7.5	1,210,000	2.612	16,666	7.421	1,226,666	1,448,124	10.033	35.00
(b) 681 g. hay, 227 g. cornstarch and 40 g. albumin egg Merck, impalpable powder										
5th	II	7.6	777,500		58,333		835,833			55.00
6th	II	7.8	453,333		29,166		482,499			29.03
8th	II	7.8	355,833		47,500		403,333			1.00
10th	II	7.8	452,500		65,833		518,333			83.87
15th	II	7.8	798,333	1.053	100,000	11.048	898,333	627,666	12.101	52.00
(c) 681 g. hay, 227 g. cornstarch and 45 g. wheat gluten flour										
13th	II	7.7	1,301,666		19,166		1,320,832			18.05
14th	II	7.7	1,920,833		87,500		2,008,333			.....
15th	II	7.8	1,691,666		21,666		1,713,332			40.63
16th	II	7.9	1,968,333		37,500		2,005,833			22.22
17th	II	7.9	2,695,833	3.555	69,166	8.630	2,764,999	1,962,666	12.185	57.90
(d) 681 g. hay, 227 g. cornstarch and 28 g. asparagin (an animo acid)										
6th	II	7.4	890,000		62,083		952,083			33.33
7th	II	7.4	666,666		61,666		728,332			44.00
8th	II	7.9	838,333		23,333		861,666			25.00
9th	II	7.8	790,833		40,000		830,833			26.31
10th	II	7.6	1,077,500	1.582	54,166	8.860	1,131,666	900,916	10.442	92.11
(e) 681 g. hay, 227 g. cornstarch and 35 g. casein										
7th	II	7.7	1,224,166		69,166		1,293,332			.....
8th	II	7.8	1,525,000		25,000		1,550,000			49.00
9th	II	7.7	950,833		13,333		964,166			46.34
10th	II	7.8	1,535,833		24,166		1,559,999			23.33
11th	II	7.7	1,662,500	2.560	10,833	5.233	1,673,333	1,408,166	7.793	40.00

TABLE 13. Effects of pregnancy

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc.	Total pctg. of vol. per cc.	Pctg. of sediment
(a) 681 g. hay and 250 g. grain										
6th	I	7.7	1,134,166		61,666		1,195,832			64.29
8th	I	7.8	1,237,500	2.201	40,833	9.411	1,278,333	1,237,082	11.612	51.22
(b) 1,362 g. hay and 500 g. grain										
5th	I	7.8	2,064,166		40,000		2,104,166			36.49
6th	I	7.8	1,593,333		25,000		1,618,333			50.79
8th	I	7.8	1,807,500		30,000		1,837,500			43.08
9th	I	7.8	1,894,166		29,166		1,823,332			50.00
12th	I	7.8	1,840,000		56,666		1,896,666			55.17
14th	I	7.8	2,385,833		80,833		2,466,666			70.21
16th	I	7.9	1,450,000		35,000		1,485,000			51.16
19th	I	7.9	1,881,666		80,833		1,962,499			24.00
21st	I	7.9	1,504,166	3.386	94,166	9.624	1,598,332	1,654,721	13.010	52.38

TABLE 14. Effects of lactation

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc.	Total pctg. of vol. per cc.	Pctg. of sediment
(a) 1,362 g. hay and 500 g. grain										
3rd	I	7.8	2,361,666		62,500		2,424,166			74.47
13th	I	7.7	2,012,500		40,000		2,052,500			51.85
14th	I	7.7	2,030,833		63,333		2,094,166			62.50
17th	I	7.8	2,775,833		59,166		2,834,999			60.53
22nd	I	7.8	2,773,333		66,666		2,839,999			73.33
27th	I	7.6	2,740,833		48,333		2,789,166			85.45
35th	I	7.7	2,566,666		63,333		2,629,999			40.00
45th	I	7.8	1,822,500		44,166		1,866,666			28.00
50th	I	7.8	2,755,000		71,666		2,826,666			31.25
55th	I	7.8	1,314,166		73,333		1,387,499			44.44
62nd	I	7.8	2,150,833		71,666		2,222,499			51.16
71st	I	7.9	1,898,333	4.207	35,000	10.699	1,933,333	2,325,138	14.906	18.61
(b) Food not entirely consumed. Kid eating some of the grain.										
78th	I	7.9	1,049,166		17,500		1,066,666			15.09
83rd	I	7.6	1,590,000		25,833		1,615,833			28.52
90th	I	7.9	1,753,333		38,333		1,791,666			41.03
97th	I	7.9	1,690,000		32,500		1,722,500			20.51
104th	I	7.8	1,277,500		39,166		1,316,666			47.22
112th	I	8.0	2,425,833	3.027	60,833	6.554	2,486,666	1,666,666	9.581	37.04
(c) Kid weaned. Slight milkings.										
119th	I	8.0	1,765,000		18,333		1,783,333			35.90
125th	I	8.0	1,648,333	3.167	99,166	10.788	1,747,499	1,765,416	13.955	56.67

the 113th day. Following this separation, however, the mother goat was milked a little each day to relieve the distention of the udder. The milkings were gradually decreased until she was milked no more after the 126th day (table 14-c).

Effects of cessation of lactation. As Goat I had not been eating all the food given her, the amount was decreased, as indicated in table 15. It is interesting to note that, previous to the cessation of the lactation period, she had preferred the grain to the hay, but after drying up she left the grain to eat the hay first. Although she was eager to receive food, the amount of hay and grain consumed fell off considerably. With the loss of appetite, and the decrease in the amount of food eaten, the infusorian numbers dropped decidedly, as table 15 indicates.

TABLE 15. *Effects of cessation of lactation*

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc.	Total pctg. of vol. per cc.	Pctg. of sediment
(a) 200 g. hay and 100 g. grain										
7th	I	8.0	550,000		833		550,833			13.83
14th	I	7.7	693,333	1.154	48,333	4.514	741,666	646,249	5.668	64.29
(b) 200 g. hay and 400 g. grain										
3rd	I	7.6	737,500		7,500		745,000			36.58
11th	I	7.7	1,463,333	2.042	12,500	1.836	1,475,833	1,110,416	3.878	24.14

Weight of Goats

The goats were weighed to determine whether an increase in protozoan numbers accompanied an increase in weight. The weights were not taken more frequently, as it was clearly apparent that they varied very little. Table 16 shows some weights of the goats.

TABLE 16. *Weights of the goats*

Date	11-18-29	12-20-29	1-24-30
Goat I	60 pounds		
Goat II	47 pounds	50 pounds	47½ pounds
Goat III	60 pounds	60 pounds	55 pounds

Because of her pregnant condition, Goat I was weighed only once. During the period between the first and second weighings, when Goat II gained three pounds, the infusoria numbers rose and fell as is shown in table 5, sections c to k, inclusive, the first weighing taking place at the close of section c, and the second after section k. Goat III, weighed after the feeding indicated in table 5-e, had no increase in weight at the second weighing, but the infusoria numbers rose considerably (table 5-f, h and k). The drop in weight in the two goats on 1-24-30 was due to the fact that

they had been starved for the preceding two days. With the reinstatement of food, they returned to their former weights, which remained about constant, until the illness of Goat III in May, 1930, when he lost weight and was discarded. It was deemed unnecessary to weigh Goats II and III after the last weighing in the table, as there was in both animals no variation in weight apparent enough to be of any importance. Except for the decrease in numbers of protozoa accompanying the loss of weight after the period of starvation, there seemed to be no correlation between the weight of the goats and the numbers of infusoria in these full-grown animals.

Effect of pH

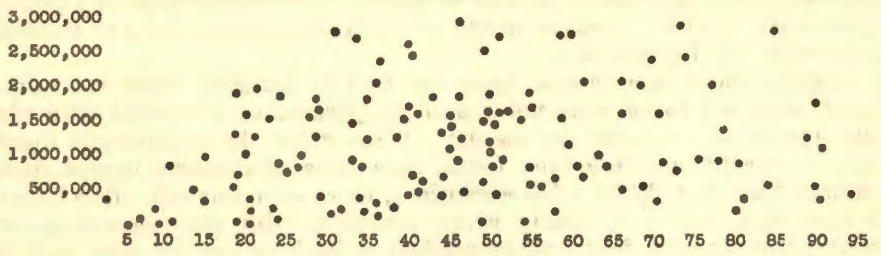
Ferber (1929, b) stated that the hydrogen ion concentration of the rumen content plays a rôle in the development and maintenance of the infusorian fauna. Under normal conditions and infusoria numbers, he observed very slight variations of the pH around 7.9, and, with a lowering of the pH to even so slightly acid as 6.9, he found a heavy depression in the numbers of infusoria. With the three goats used in these experiments, however, 66 per cent of the determinations made fell within the range of 7.6 to 7.8, with 50 per cent of the total at 7.7, with only one-fourth of the remainder of the samples as high as 7.9 to 8.2, and three-fourths 7.5 down to 6.7. This would indicate a slightly lower normal average pH for these three goats than for those used by Ferber, or 7.7 for normal pH in these experiments. For the most part, as is seen by glancing over the tables, the pH and the numbers of infusoria are in general agreement with the correlations as stated by Ferber, but there are a few noticeable discrepancies. For example, in table 2-a, the numbers increased materially in Goat III, while the pH was down to 7.2, and in table 3, with Goat I, the highest levels during the entire series of experiments were reached at a pH of 6.9 and 7.0. However, the sudden drop in numbers immediately following may have been due to the low pH. Again, in table 5-f and g, the numbers were high in all three goats, with a pH as low as 7.2 and 7.0. It might seem, from tables 3 and 5-f and g, that the grain mixture in excess caused the drop in the pH level, as the other sections in table 5 show higher pH levels, with grain, but with more hay in proportion. Table 10 indicates a similar condition with the addition of cracked corn to the diet, for the numbers were high, with a lowered pH. The great drop in numbers and the slightly acid pH for Goat III shown in table 12-b coincide with Ferber's results (1928 and 1929, b). During pregnancy and lactation, the pH remained up. (See tables 13 and 14).

In discussing Ferber's experiments regarding the effect of the pH upon the presence of the infusoria, Mangold (1929, b) stated that the disappearance of the infusoria could not be due to variations in the pH, since, during the starvation experiments with the decrease in infusorian numbers, the pH varied only slightly. Also, with renewed feeding after the starvation periods, the infusoria regained their former numbers by the same pH as that under which they had disappeared. This fact was also apparent in our experiments. (See table 7).

Effect of Density

Ferber also stated (1929, b) that the density of the rumen contents played a rôle in governing the infusoria numbers. He designated the various densities of the rumen samples as "normal", "thick", "thin", etc., and found an apparent agreement between the density of the rumen contents and the numbers of infusoria. While the tables (7-on) for these experiments show an occasional agreement between the percentage of sediment and the infusorian numbers, the discrepancies are so numerous that it hardly seems feasible or possibble to make any such statement as that of Ferber. For example, in table 8-a, the highest percentages of sediment accompanied the lowest infusorian numbers, and, in section b, with greatly increased counts of protozoa, the samples did not increase in density, but were low in percentage of sediment; also table 12, for the most part, bears out the same lack of agreement. Tables 13 and 14, showing the conditions during pregnancy and lactation, do not indicate the "very thick" densities, according to the numbers of protozoa, that one would expect to find from Ferber's conclusions. An inspection of the correlation chart, table 17, shows an apparent lack of any great degree of correlation between the percentage of sediment and the numbers of infusoria in 132 samples of rumen contents taken from all three goats over a period of five months. It is to be concluded, therefore, that the numbers of protozoa present are not to be correlated with the amount of sediment in the rumen contents.

TABLE 17. *Correlation Chart: percentage of sediment, abscissa; numbers of Infusoria, ordinate*



Winogradowa-Fedorowa and Winogradoff (1929) did not consider the percentage of sediment any indication of the amount of the protozoan fauna. They state that they have found from examinations of the rumen contents of slaughtered animals, that the anterior portion of the rumen contents is more fluid than the posterior. They point out that, since large food particles will not pass through the suction tube while samples are being taken from living animals, only the more fluid portion of the contents is withdrawn. The result is that the sample does not give a true average of the density of the contents. Accordingly, after taking samples of the rumen content of living animals, they added a definite quantity of water to the stomach and took more samples after a stated interval of time. By

a series of mathematical computations they then determined the average density of the rumen content. Their tables showing the differences obtained by the two methods indicate very little variation, however.

Mangold (1929, b), took exception to their results in that they compared the rumen contents of living animals with those of slaughtered ones. In his judgment Ferber's methods were not open to severe censure, as the samples taken were frequently very thick and therefore could not represent merely the more fluid portion of the rumen content. He mentioned further that there is a thorough mixing of the rumen content in the living animal, and that the separation into thicker and thinner portions appears only as a post-mortem variation. He did express the opinion, however, that there is not always a parallel between the number of rumen infusoria and the density of the rumen contents.

Rate of Division of Infusoria

In 1929, Ferber and Winogradowa-Fedorowa published the results of investigations on the division rate of the infusorian fauna in the rumen of sheep and goats. They reasoned that since under normal conditions the protozoan numbers remained fairly constant, the loss from the forms passed on from the rumen with the food material for further digestion must be replaced by the division of some of the remaining forms. In the course of their experiments, they computed an average of seven per cent division forms to the total number counted, and this average remained constant even for two counts per day, taken both before and after the consumption of food by the host animal. Their reasoning, however, is faulty, for the division rate of rumen infusoria is still unknown; and, furthermore, it is quite unlikely that a percentage of a population observed at any moment in the act of dividing represents the amount of reproduction for that day.

In the above experiments, from the first of January, 1930, the numbers of dividing forms were noted and the percentage computed with the total number of the count for the day. Throughout the experiments there were practically no Diplodinia found in a state of division, except in a few rare instances. With a few exceptions, there were some dividing forms of Entodinia found at nearly every counting. But the percentage of recognizable division forms never reached as high as one per cent in any case, and were more often below 0.5 of one per cent than above. Even during the periods of pregnancy and lactation, when a higher percentage of division forms might be expected in accord with the increased fauna, there was little increase in the rate of division, and the total number of forms dividing never amounted even to one per cent of the total number of forms found. It is difficult to estimate the amount of division in this way, however, for only somatic indications of division are recognizable. Stained slides would be necessary to make even fairly accurate estimates of the numbers of dividing forms.

Volume of Protozoa

The average volume of a single specimen for each of the two genera was determined by the displacement of water, as previously described.

From this, the average percentage of volume for each genus was computed for the average with each feed, as is shown in the tablse. It is of interest to note that the average volume of *Diplodinium multivesiculatum* is approximately one hundred times as great as that of an average Entodinium, the volume of a single average specimen of the latter being 18,560 cubic microns, and that of a single specimen of the former 1,836,320 cubic microns. The average percentage of volume per cc. of rumen contents for each feed is indicated in the tables, and shows that, despite the higher numbers of Entodinia present, the main volume of the infusoria is represented by the Diplodinia. The highest total percentage of volume was obtained in the case of Goat I on a feed of green alfalfa and 1,000 g. of grain, when it reached 27.274 per cent (see table 2-b). The peak reached by the infusoria numbers was for Goat I on the 6th day of feed with 1,000 g. of grain (table 3). The per cent of volume of the infusoria for that day amounted to 28.036 per cent, of which 13.958 per cent represents the volume of Entodinia, and 14.078 per cent that of the Diplodinia. The highest percentage of volume was obtained from Goat I on the 26th day of feed with green alfalfa and 1,000 g. of grain (table 2-b), when the numbers of Diplodinia reached the highest peak throughout the entire course of the experiments. On that day, the volume of the Diplodinia alone reached 31.83 per cent, which, with 7.24 per cent volume of Entodinia, gave a total percentage of volume of 39.07 per cent.

DISCUSSION AND SUMMARY

The results obtained in the feeding experiments indicate very clearly that the amount of the infusorian population in the rumen, whether measured by population or by volume, can be manipulated through the food of the animal regardless of its special physiological condition. The numbers of infusoria in adult goats not receiving food fall off rapidly, as has been shown previously by other workers. On a hay diet alone the numbers are comparatively low. The population is augmented by the addition of a small amount of grain to the ration. With each succeeding increase in the grain allotment there is a subsequent rise in numbers of infusoria. Similarly, and contrary to Ferber's experience, it has repeatedly been shown that cornstarch used as a supplementary feed with hay will result in more than a doubling of the numbers of infusoria with a diet of hay alone. The addition of small amounts of suitable materials rich in animal protein, such as dried blood serum, to the hay and starch constituents of the diet will stimulate the reproduction of the infusoria exceedingly.

Parenthetically, it may be noted that it is not known whether these materials regulate the numbers of infusoria directly or indirectly. All attempts by investigators up to the present time to grow holozoic protozoa in purely liquid media have failed. It is possible that an increase in number of infusoria is dependent upon the preliminary development of bacteria at the expense of the protein materials in solution. Nevertheless, whether the development of the protozoan fauna is directly or indirectly conditioned by the food materials introduced into the rumen with the food, the end result is the same. Starch grains are ingested by the infusoria and digested directly by them, as shown by Trier. It has been shown by Dobell that starch stimulates the growth of *Endamoeba histolytica* in cul-

ture. It is not unlikely that other elements in the grain are ingested as solid particles and digested in the endoplasm of the infusoria.

It will be noted that there are many points of agreement in our results with those obtained in Mangold's laboratory. Under conditions of normal feeding there is a fairly constant infusorian fauna of around 1,000,000 organisms per cc. of rumen contents. The numbers fall off rapidly during starvation, and rise with resumed feeding until after about nine days the normal level is attained again. During pregnancy and lactation the numbers are much higher. Upon cessation of lactation, the numbers become much less. Granting, in general, that the data of Ferber and his co-workers are fundamentally correct, how should they be interpreted? Does a concomitant increase or decrease in numbers of infusoria in the stomach of a ruminant at periods of high protein utilization imply causation?

The fallacy of the conclusion that the infusoria are symbionts lies in this very point. It is undoubtedly true that at times of greater protein utilization by the host—such as during growth, pregnancy, or lactation—the numbers of infusoria in the rumen do increase; and conversely, at times of lesser protein utilization—such as during maturity, old age, or after the cessation of lactation—the numbers do decrease. But do these phenomena, as Ferber and Mangold deduce, indicate a condition of symbiosis or mutual aid between the host and parasite? A conclusion to the affirmative, without other more pertinent evidence, could result only from a process of reasoning of the *post hoc, ergo propter hoc* type. It is quite fitting to inquire whether there may not exist a factor other than the physiological condition of the host which will explain the rise and fall of the protein metabolism of the host.

If our results mean anything at all, they indicate that the numbers of infusoria are dependent directly upon the amount and kind of food which the host consumes. The artificial manipulation of the feed of mature, male goats can bring about numbers of infusoria corresponding to those in growing, pregnant, or lactating animals; or on the other hand, similar to those in "drying up", or aged animals.

It is a well known fact, and one so stated in standard works on nutrition, that growing, pregnant, or lactating animals consume more concentrated foods, when these are available, than they would otherwise. This was very evident in the case of Goat I, which became very greedy for concentrates at the onset of and during pregnancy, and also during lactation. At the time of cessation of lactation the grain ration was only partially consumed and much less relished.

Here, then, an explanation of the behavior of the infusorian population at various times in the life history of the ruminant seems to present itself. It is the increase and change in the appetite which results in the animal eating more and richer feeds which leads to an increase in number of rumen infusoria. This supplies an intelligible *modus operandi* for the sequence of events in the infusorian life of the rumen. It is evident to us, after reading most carefully the works of Ferber and of Mangold, that they did not have exactly this factor in mind, for Mangold states explicitly that the mechanism controlling the relationship between physiological need and numbers of infusoria is unknown. The following translation is from

page 176 of his work "Die Verdauung der Wiederkäuer" (1929, b):—"There must still be solved the question what physiological changes in the rumen of ruminants there are which, as soon as its protein metabolism ascends, cause the increase in the infusoria number, and conversely, what physiological factors in the lowering of the protein metabolism condition the decrease of the infusoria number." And on the same page follows this sentence:—"Experiments seem to have proved that the primary variations always start from the host animal, and the changes of infusoria number represent the secondary appearance."

In face of the admission that the nature of the physiological factors which regulate the numbers of infusoria in the rumen is unknown, any pronouncement to the effect that the relationship between host and parasite is one of symbiosis would seem premature and unwarranted. A critical study of the influence of diet upon the numbers of infusoria shows that the food plays a major rôle in conditioning the numbers of infusoria. A more sound philosophy of the nature of the association would be that it is one of commensalism. The infusoria live up to the margin of subsistence provided for them in the rumen. When the food is scanty or composed largely of plant fiber, the numbers are comparatively small. When there is an abundance of rich and easily digested food present in the rumen, the numbers of infusoria increase tremendously. The infusoria are thus "fair weather friends" of the ruminant, for they abound in numbers when there is an abundance of nutritious food. This philosophy is much more logical than one which implies that the micro-organisms in question unlock reserves of proteins or other food materials difficult to digest by first converting these materials into the substance of their own bodies and then sacrificing themselves to the digestion of the host.

This conclusion that the relationship is one of commensalism agrees with the results obtained by Becker, Schulz and Emmerson (1930) and by Becker and Everett (1930), who compared the digestion and growth in infusoria-free and infected lambs and goats. The comparison was made possible by the development by Becker (1929) of a method of freeing the rumen of infusoria through the administration of a copper sulphate solution.

CONCLUSIONS

1. The amount and kind of food consumed are of prime importance in the regulation of the numbers of rumen infusoria.
2. There is no correlation, in adult goats, between the infusoria numbers and the weights of the goats.
3. The percentage of sediment in a sample of rumen contents is no indicator of the amount of the protozoan fauna.
4. The hydrogen ion concentration of the rumen contents evidently has some influence upon the fauna, but it is not a prime factor.
5. The physiological condition of the host regulates the amount of infusoria present in the rumen only in so far as it may affect hunger and appetite.
6. The reactions of the infusoria to conditions in the rumen regulated by the kind and amount of food consumed by the host connote commensalism rather than symbiosis.

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